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PATENT COOPERATION TREATY

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-06- 20 2000

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JUN 19 2000

P.M. 7 8 9 10 11 12 1 2 3 4 5 6

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

OGILVY RENAULT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
Van Zant & Associates
Attn. Van Zant, Joan M.
77 Bloor Street West
Suite 1407
Toronto, Ontario M5S 1M2
CANADA

DUE ON AUG 13 2000
AMEND CLAIMS

Date of mailing *(day/month/year)* 13/06/2000	
Applicant's or agent's file reference P36PCT5	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/CA 00/ 00144	International filing date *(day/month/year)* 11/02/2000
Applicant BIOCHEM PHARMA INC. et al.	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Sandra De Jong-van Dam

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international pbulication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the l tter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

RECEIVED SWABEY
-06- 11 2001
PCT OGILVY RENAULT

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
JVZ
SWABEY OGILVY RENAULT
1981, Avenue McGill College
Bureau 1600
Montréal, Québec H3A 2Y3
CANADA

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	05.06.2001
Applicant's or agent's file reference P36PCT5	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00144	11/02/2000	11/02/1999

Applicant
BIOCHEM PHARMA INC. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Cardenas, C Tel.+31 70 340-3370

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P36PCT5		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00144	11/02/2000	11/02/1999
International Patent Classification (IPC) or national classification and IPC C12P41/00		
Applicant BIOCHEM PHARMA INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
30/08/2000	05.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Montero Lopez, B Telephone No. +31 70 340 3739

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00144

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-69 as originally filed

Claims, No.:

1-16 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-16
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-16
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-16
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
s e separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following document:

D1: US-A-5 276 151 (DENNIS C. LIOTTA) 4 January 1994 (1994-01-04)

1. The underlying application relates to a process for stereoselectively producing a dioxolane nucleoside analogue by selectively hydrolysing an anomeric ester mixture and subsequently replacing the functional group at the C_4 position with a purine or pyrimidine. Nowhere in the state of the art has been such method used for the production of stereospecific dioxolane nucleosides analogues. Claims 1-16, therefore, are novel and comply with the requirements of Article 33(2) PCT.

2. Document D1, which is considered to represent the most relevant state of the art, discloses (cf. col. 11, line 31 - col. 13, line 46) a process for the resolution of racemic mixtures of dioxolane nucleosides by the enzymatic hydrolysis of the nucleoside esters at position C_5. No hint exists in the state of the art which would allow the skilled person to devise such a process as disclosed in claims 1-16, in which the purine or pyrimidine base is introduced subsequently to the anomeric separation. Consequently, the subject-matter of claims 1-16 involves an inventive step and complies with the requirements of Article 33(3) PCT.

Re Item VII

Certain defects in the international application

1. The vague and imprecise statement in the description on the paragraph bridging pages 68 and 69 implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity (Article 6 PCT) when used to interpret them (see also the PCT Guidelines, III-4.3a).

2. General statements in the description which imply that the extent of protection may be expanded in a not precisely defined way are not allowable. The applicant is therefore kindly requested to delete the expression "WO/97/21706 is incorporated herein fully by reference" from page 29, first paragraph.

3. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the document D1 is not mentioned in the description, nor is this document identified therein.

Re Item VIII

Certain observations on the international application

1. The terms "analogue" and "derivative" used in claims 1, and 14-16 are vague and unclear and leave the reader in doubt as to the meaning of the technical features to which they refer, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

2. The internal designations used to define enzymes ESL-001-02 used in claims 1 and 10 and ESL-001-05 used in claims 1 and 13 are meaningless and do not constitute characterizing technical features which define the intended product. This introduces an unclarity in the scope of claims 1, 10 and 13 contrary to Article 6 PCT.

3. The expression "second mixture" used in claim 16 introduces an unclarity contrary to Article 6 PCT in the scope of the claim since the claim is silent about the composition or identity of any "first" or "second" mixtures.

4. Analogously, the expressions "starting material" and "product" used in claims 2-9 render the scope of these claims unclear contrary to the requirements of Article 6 PCT. Since the claimed process consists in the preferential hydrolysis of a component of a mixture, resulting in another mixture of different composition, it is not clear what the identity of such "starting material" and "product" is meant to be.

PATENT COOPERATION TREATY

SWABEY OGILVY RENAULT
McGILL COLLEGE
RECEIVED
NOV 2 4 2000

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
SWABEY OGILVY RENAULT
1981, Avenue McGill College
Bureau 1600
Montréal, Québec H3A 2Y3
CANADA

A.M. 7 8 9 10 11 12 1 2 3 4 5 6 P.M.

DUE ON FEB 2 1 2001

Short Term

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	21.11.2000
Applicant's or agent's file reference: P36PCT5	**REPLY DUE** **within 3 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00144	11/02/2000	11/02/1999

International Patent Classification (IPC) or both national classification and IPC
C12P41/00

ENTERED Sec. 4

Applicant
BIOCHEM PHARMA INC. et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☐	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain document cited
VII	☒	Certain defects in the international application
VIII	☒	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 11/06/2001.

Name and mailing address of the international preliminary examining authority:
European Patent Office - P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040 Tx: 31 651 epo nl
Fax: +31 70 340 - 3016

Authorized officer / Examiner
Montero Lopez, B

Formalities officer (incl. extension of time limits)
Sinanovic, E
Telephone No. +31 70 340 2672

I. Basis of th opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".)*:

Description, pages:

1-69 as originally filed

Claims, No.:

1-16 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item VII

Certain defects in the international application

1. The vague and imprecise statement in the description on the paragraph bridging pages 68 and 69 implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity (Article 6 PCT) when used to interpret them (see also the PCT Guidelines, III-4.3a).

2. General statements in the description which imply that the extent of protection may be expanded in a not precisely defined way are not allowable. The applicant is therefore kindly requested to delete the expression "WO/97/21706 is incorporated herein fully by reference" from page 29, first paragraph.

Re Item VIII

Certain observations on the international application

1. The terms "analogue" and "derivative" used in claims 1, and 14-16 are vague and unclear and leaves the reader in doubt as to the meaning of the technical features to which they refer, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

2. The internal designations used to define enzymes ESL-001-02 used in claims 1 and 10 and ESL-001-05 used in claims 1 and 13 are meaningless and do not constitute characterizing technical features which define the intended product. This introduces an unclarity in the scope of claims 1, 10 and 13 contrary to Article 6 PCT.

3. The expression "second mixture" used in claim 16 introduces an unclarity contrary to Article 6 PCT in the scope of the claim since the claim is silent about the composition or identity of any "first" or "second" mixtures.

4. Analogously, the expressions "starting material" and "product" used in claims 2-9 render the scope of these claims unclear contrary to the requirements of Article 6 PCT. Since the claimed process consists in the preferential hydrolysis of a component of a

mixture, resulting in another mixture of different composition, it is not clear what the identity of such "starting material" and "product" is meant to be.

09/890243

Copy for the Elected Office (EO/US) PCT/CA00/00144

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

To:

SWABEY OGILVY RENAULT
Suite 1600
1981 McGill College Avenue
Montréal, Québec H3A 2Y3
CANADA

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day/month/year) 09 August 2001 (09.08.01)	
Applicant's or agent's file reference P36PCT5	**IMPORTANT NOTIFICATION**
International application No. PCT/CA00/00144	**International filing date** (day/month/year) 11 February 2000 (11.02.00)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [ ] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
MCGILL UNIVERSITY 845 Sherbrooke Street West Montreal, Quebec H3A 1B1 Canada	CA	CA
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:
New applicant for all designated States except US.

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Eugénia Santos (Fax 338.87.40)
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994) 004204065

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 04 October 2000 (04.10.00)	
International application No. PCT/CA00/00144	**Applicant's or agent's file reference** P36PCT5
International filing date (day/month/year) 11 February 2000 (11.02.00)	**Priority date** (day/month/year) 11 February 1999 (11.02.99)
Applicant CIMPOIA, Alex et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

30 August 2000 (30.08.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Charlotte ENGER
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12P 41/00

A1

(11) International Publicati n Number: WO 00/47759

(43) International Publication Date: 17 August 2000 (17.08.00)

(21) International Application Number: PCT/CA00/00144

(22) International Filing Date: 11 February 2000 (11.02.00)

(30) Priority Data:

60/119,756	11 February 1999 (11.02.99)	US
60/119,885	12 February 1999 (12.02.99)	US

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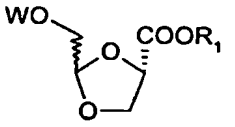
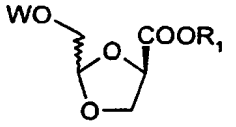


(A)



(B)

(57) Abstract

The present invention provides a process for making stereochemically pure dioxolane nucleoside analogues. The process includes the use of hydrolytic enzymes for separating β and α anomers from an anomeric mixture represented by formula (A) or formula (B) wherein W is benzyl or benzoyl; R_1 is selected from the group consisting of C_{1-6} alkyl and C_{6-15} aryl.

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STEREOSELECTIVE SYNTHESIS OF NUCLEOSIDE ANALOGUES

FIELD OF THE INVENTION

The present invention relates generally to a novel method for the preparation of nucleoside analogues and their precursors and more particularly to a method of preparing a nucleoside analogue by the use of specific enzymes to stereoselectively produce dioxolane nucleoside analogues or their precursors.

BACKGROUND OF THE INVENTION

The pharmacological activity of pharmaceutical compounds (drugs) depend mainly on their interaction with biological matrices (drug targets), such as proteins (receptors and enzymes), nucleic acids (DNA and RNA) and biomembranes (phospholipids and glycolipids). All these drug targets have complex three-dimensional structures which are capable of binding specifically to the drug in only one of the many possible arrangements in the three-dimensional space. It is the three-dimensional structure of the drug target that in part, determines which of the potential drug is bound within its cavity and with what affinity.

The spatial arrangement of atoms in an asymetric molecule is termed chirality. Chirality results in the creation of stereoisomers. Stereoisomers are compounds with identical chemical composition and atom connectivity (i.e. same constitution), but different arrangements of the atoms in space (i.e. different configurations). Stereoisomers are classified according to the number of

chiral centers in each molecule and the spatial arrangement of the chiral center.

Chiral centers of organic molecules include chiral carbon atoms which have four different substituents connected thereto and arranged in a generally tetrahedral configuration. Another type of chiral center is a chiral plane oriented along a rigid C=C bond that has at least two different substituents connected to the remaining four bond positions in that arrangement.

The chirality of molecules that are the subject of the present application refer to chirality created by chiral atoms and not chiral bonds. The following discussions will be limited to chirality created at one or more chiral carbon atoms which have four different substituents bound to each of the four different binding sites of the carbon.

When a molecule has a single chiral carbon, there are two stereoisomers that are mirror images of each other. This pair of isomers is termed enantiomers or an enantiomeric pair. When there are two chiral carbon atoms, there are four stereoisomers and two pairs of mirror images or enantiomers. A stereoisomer which is not a mirror image of another stereoisomer is a diastereoisomer.

One type of stereochemical distinction relates to cyclic sugars or analogues of cyclic sugars. Cyclic sugars can be designated as a particular anomer depending upon the stereochemical configuration.

The term "anomer" designates the spatial arrangement of cyclic sugars or analogues or derivatives thereof that

have two chiral centers in a five or six member ring. The anomeric designation defines the relative configuration of the two chiral centers relative to a hypothetical plane defined by the ring. The chiral centers typically have two substituents outside the ring. One substituent is a H. The other substituent is a larger moiety such as a hydroxyl, methoxyl, purine or pyrimidine base, carboxyl, etc.

When the two larger constituents on each chiral center are on the same side of the plane in the ring, they are defined as a β-anomer (cis-isomer). When two larger moieties are on opposite sides of the plane in the ring, they are defined as the α-anomer (trans-isomer). An anomer is a type of diastereoisomer.

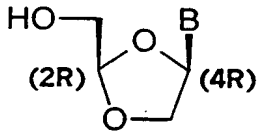
Because chirality may affect biological activity or toxicity, it is important from the point of view of drug development to evaluate the physiological effect of each isomer. Frequently, one stereoisomer is considerably more active than the other. In other situations, the non-active isomer may inhibit the activity of the more active form. In some instances, the less preferred stereoisomer may be equally potent but have greater toxicity than the preferred stereoisomer. In each of these instances, the therapeutic effect of a drug can be increased if the single most preferred stereoisomer is administered in higher purity.

The current trend in the drug markets reflects a greater use of single stereoisomer drugs. The sales of single stereoisomer drugs have increased considerably. In 1995, sales of single stereoisomer drugs reached $61 billion

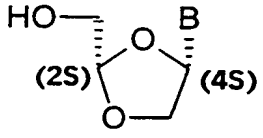
worldwide. In the year 2000, the annual worldwide sales are expected to reach $90 billion.

An important class of pharmacological agents relate to 3'-oxa-substituted 2',3'-dideoxynucleoside analogues ("dioxolane nucleoside analogues"). These compounds have two chiral centers corresponding to the substituted carbons 2 and 4 of the dioxolane ring (C2 and C4 respectively). Thus each compound can exist as four different stereoisomers depending on the position of both substituents with respect to the dioxolane ring.

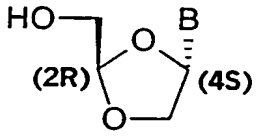
The stereoisomers of a dioxolane nucleoside analogue are represented by the following diagrams where the letter B represents a purine or pyrimidine base or an analogue or derivative of a purine or pyrimidine base as defined herewith.



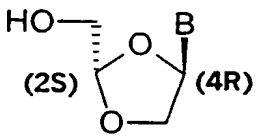
β-D



β-L



α-D



α-L

For the purpose of consistency, the same stereochemical designation is used when the methyloxyl moiety or the base moiety is replaced with another substituent group.

The C2 carbon in each of the above formula is the carbon atom in the ring that connects the methyloxy group to the dioxolane ring. The C4 carbon is the carbon atom in each of the above formula that connects the base (B) substituent to the dioxolane ring.

The four stereoisomers represented above correspond to two pairs of enantiomers. The β-anomers represent one set of enantiomers, the β-L enantiomer and the β-D enantiomer. The α-anomers represent the other set of enantiomers, the α-L enantiomer and the α-D enantiomer.

Compounds with D-configuration have an outward directed methyloxy group when the ring is oriented in the plane of the page with the oxygen in the first position (O3) at the top of the page with the carbon in the two position (C2) on the left side as illustrated above. This is also represented by the designation (2R). Compounds having an L-configuration has inward directed methyloxy group when the ring is oriented in the plane of the page with the O3 oxygen at the top of the page with the C2 carbon on the left side as illustrated above. This is also represented by the designation (2S).

A variety of dioxolane nucleoside analogues have been identified to have antiviral and anticancer activity. For example, 9-(β-D-2-hydroxymethyl-1,3-dioxolan-4-yl)-2-aminopurine (β-D-DAPD) and its metabolite 9-(β-D-2-hydroxymethyl-1,3-dioxolan-4-yl)-guanine (β-D-DXG) have been reported to have potent and selective activity against human immunodeficiency virus (HIV) and hepatitis B virus (HBV) (Rajagopalan et al., Antiviral Chem. Chemother.,1996, 7(2), 65-70) Similarly, 1-(β-L-2-hydroxymethyl-1,3-dioxolan-4-yl)-thymine (Dioxolane-T)(Norbeck et al., Tetrahedron Lett., 1989,30, 6263-66) possess anti-HIV and anti-HBV activity. 1-(β-L-2-hydroxymethyl-1,3-dioxolan-4-yl)-cytidine (β-L-OddC) (Bednarski et al., Bioorg. Med. Chem. Lett., 1994, 4,

2667-72) was discovered to have potent anti-tumor activity towards human prostate as well as renal carcinoma (Kadhim et al., Can. Cancer Res., 57(21),4803-10, 1997). β-L-OddC is the first nucleoside analogue with an L-configuration shown to have anticancer activity. Since stereoisomers of dioxolane nucleosides usually have different biological activities and toxicity, obtaining the pure therapeutically active isomer becomes crucial.

Chiral synthetic methods have improved over the past several years with respect to synthetic techniques that result in single stereoisomer compounds. However, there is a present need to find novel synthetic methods which can be widely used to form a particular stereoisomer with greater efficiency and purity.

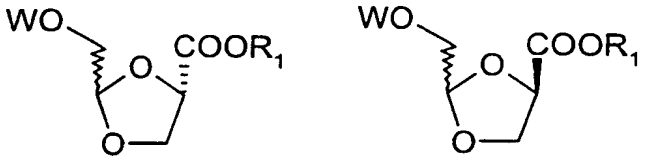
For example, for many years a person of ordinary skill in the art could use enzymes to separate enantiomers of dioxolane compounds. However, it is not known in the art how to produce a dioxolane nucleoside analogue using a step of separating an anomeric mixture of certain dioxolane precursors using enzymes to produce a stereochemically pure end product with greater efficiency and purity.

Because stereochemically pure dioxolane nucleosides are an important class of compounds due to their known antiviral and anticancer activity, there is a need for other inexpensive and efficient stereoselective methods for their preparation. The present invention satisfies this and other needs.

SUMMARY OF THE INVENTION

The present invention provides a novel process for making dioxolane nucleoside analogues with a high degree of steric purity, greater efficiency and higher yields.

Specifically, the present invention provides a process for making dioxolane nucleoside analogues with a high degree of steric purity which includes the use of certain hydrolytic enzymes for separating β and α anomers from an anomeric mixture represented by the following formula A or formula B:



(A) (B)

In the above formula, W is benzyl or benzoyl; R_1 is selected from the group consisting of C_{1-6} alkyl and C_{6-15} aryl.

The process involves the step of hydrolyzing the mixture of compounds represented by formula A and formula B with an enzyme selected from the group consisting of cholesterol esterase, ESL-001-02, horse liver esterase, bovine pancreatic protease, α-chymotrypsin, protease from *Streptomyces caespitosis*, substilisin from *Bacillus licheniformis*, protease from *Aspergillus oryzae*, proteinase from *Bacilius licheniformis*, protease from *Streptomyces griseus*, acylase from *Aspergillus melleus*, proteinase from *Bacillus subtilis*, ESL-001-05, pronase protease from *Streptomyces griseus*, *lipase* from *Rhizopus arrhizus*, lipoprotein lipase from *Pseudomonas* species

type B, lipase from *Pseudomonas cepacia* and bacterial proteinase. The process stereoselectively hydrolyses predominantly one anomer to form a product where R_1 of formula A and formula B is replaced with H. The other anomer remains substantially unhydrolysed. The process also comprises separating the hydrolyzed product from unhydrolysed starting material.

The process of one embodiment further includes the step of stereoselectively replacing the functional group at the C4 position of the dioxolane (e.g. $COOR_1$) with a purinyl, pyrimidinyl or analogue or derivative thereof to produce a dioxolane nucleoside analogue that has a high degree of steric purity.

According to one embodiment of the invention, the step of hydrolyzing results in a starting material having an anomeric purity of at least 80%. According to another embodiment, the step of hydrolyzing results in a starting material having an anomeric purity of at least 90%. In yet another embodiment, the step of hydrolyzing results in a starting material having an anomeric purity of at least 95%. In an additional embodiment, the step of hydrolyzing results in a starting material having an anomeric purity of at least 98%.

According to one embodiment of the invention, the step of hydrolyzing results in a product having an anomeric purity of at least 80%. According to another embodiment, the step of hydrolyzing results in a product having an anomeric purity of at least 90%. In yet another embodiment, the step of hydrolyzing results in a product having an anomeric purity of at least 95%. In an

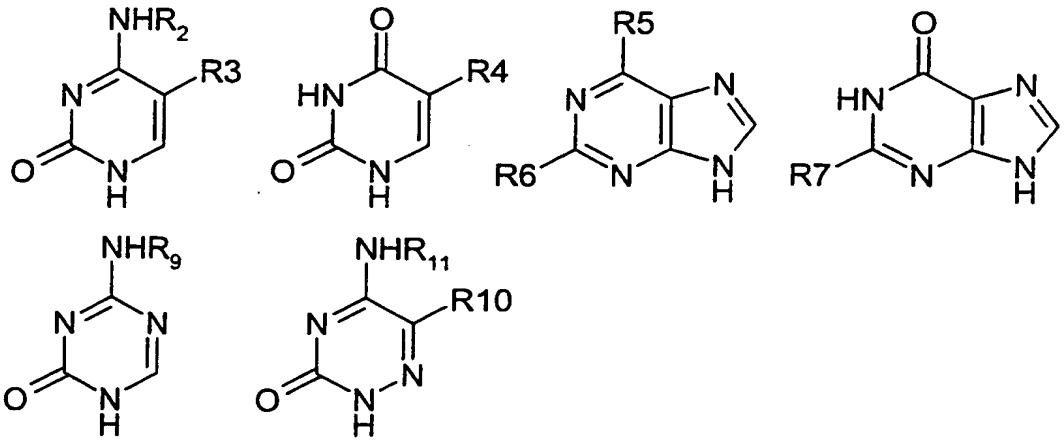
additional embodiment, the step of hydrolyzing results in a product having an anomeric purity of at least 98%.

In one embodiment of the present invention, W of formula A or formula B is benzyl and the enzyme is selected from the group consisting of cholesterol esterase, ESL-001-02, horse liver esterase, bovine pancreatic protease, α-chymotrypsin, protease from *Streptomyces caespitosis*, substilisin from *Bacillus licheniformis*. In another embodiment, the enzyme is α-chymotrypsin. In yet another embodiment, the enzyme is bovine pancreatic protease.

In one embodiment of the present invention, W of formula A and formula B is benzoyl and the enzyme is selected from the group consisting of protease from *Aspergillus oryzae*, proteinase from *Bacillus licheniformis*, subtilisin from *Bacillus licheniformis*, protease from *Streptomyces griseus*, acylase from *Aspergillus melleus*, proteinase from *Bacillus subtilis*, ESL-001-05, pronase protease from *Streptomyces griseus*, lipase from *Rhizopus arrhizus*, lipoprotein lipase from *Pseudomonas* species type B, bacterial proteinase, lipase from *Pseudomonas cepacia*. In another embodiment, the enzyme is selected from the group consisting of *Aspergillus oryzae protease*, proteinase from *Bacillus licheniformis*, subtilisin from *Bacillus licheniformis*, protease from *Streptomyces griseus*, pronase protease from *Streptomyces griseus*, and lipase from *Rhizopus arrhizus*. In yet another embodiment, the enzyme is selected from the group comprising *Aspergillus oryzae* and proteinase from *Bacillus licheniformis*.

In one embodiment, the β-anomer is the predominant product. In another embodiment, the α-anomer is the predominant product. In yet another embodiment, the β-L-enantiomer is the predominant product. In an additional embodiment, the β-D-enantiomer is the predominant product. In yet another embodiment, the α-L-enantiomer is the predominant product. In an additional embodiment, the α-D-enantiomer is the predominant product.

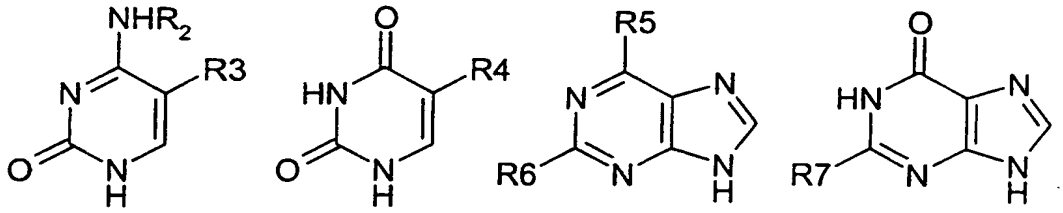
In one embodiment, the invention is a process for stereoselectively preparing a dioxolane nucleoside analogue by separating β and α-anomers from an anomeric mixture represented by formula A or formula B according to one of the above embodiments. The process further includes the step of stereoselectively replacing the functional group at the C4 position ($COOR_1$) with a purinyl or pyrimidinyl or analogue or derivative selected from the group consisting of:



In this embodiment, R_2, R_9 and R_{11} are independently selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6} acyl and $R_8C(O)$ wherein R_8 is hydrogen or C_{1-6}

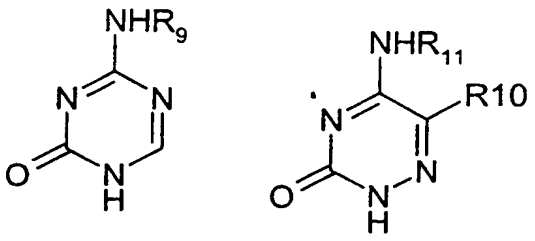
alkyl. Additionally, R_3, R_4 and R_{10} are each independently selected from the group consisting of hydrogen, C_{1-6} alkyl, bromine, chlorine, fluorine, iodine and CF_3; and R_5, R_6 and R_7 are each independently selected from the group consisting of hydrogen, bromine, chlorine, fluorine, iodine, amino, hydroxyl and C_{3-6} cycloalkylamino. The process results in the production of a stereochemical isomer of the dioxolane nucleoside analogue.

According to one embodiment, the process further includes the step of stereoselectively replacing the functional group at the C4 position ($COOR_1$) with a purinyl or pyrimidinyl or derivative selected from the group consisting of:



In this embodiment, R_2 is selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6} acyl and $R_8C(O)$ wherein R_8 is hydrogen or C_{1-6} alkyl. Additionally, R_3 and R_4 are each independently selected from the group consisting of hydrogen, C_{1-6} alkyl, bromine, chlorine, fluorine, iodine and CF_3; and R_5, R_6 and R_7 are each independently selected from the group consisting of hydrogen, bromine, chlorine, fluorine, iodine, amino, hydroxyl and C_{3-6} cycloalkylamino. The process results in the production of a stereochemical isomer of a dioxolane nucleoside analogue.

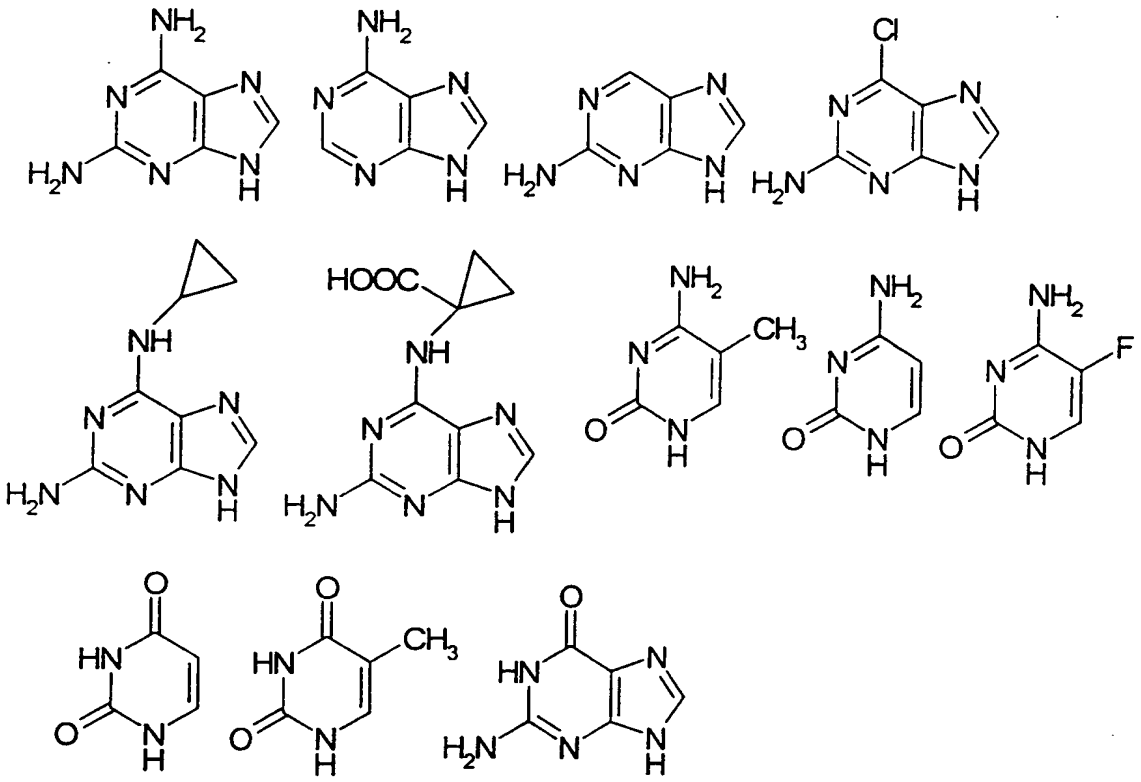
In another embodiment, the process further includes the step of stereoselectively replacing the functional group at the C4 position ($COOR_1$) with a pyrimidinyl or analogue or derivative selected from the group consisting of:



In this embodiment, R_9 and R_{11} are independently selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6} acyl and $R_8C(O)$. Additionally, R_{10} is selected from the group consisting of hydrogen, C_{1-6} alkyl, bromine, chlorine, fluorine, iodine and CF_3. The process results in the production of a stereochemical isomer of a dioxolane nucleoside analogue.

In another embodiment, the process comprises stereoselectively preparing a dioxolane nucleoside analogue by separating β and α anomers from an anomeric mixture represented by formula A or formula B according to one of the above embodiments and further comprises stereoselectively replacing the functional group at the

C4 position ($COOR_1$) with a moiety selected from the group consisting of:



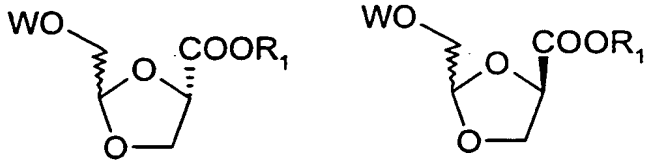
In another embodiment of the present invention, the process comprises making a dioxolane nucleoside analogue by separating a compound according to formula A or formula B. According to this embodiment, the process includes stereoselectively replacing the R group with a purinyl or pyrimidinyl moiety or analogue or derivative thereof by acylating the second mixture to produce an acylated second mixture. This embodiment also includes the step of glycosylating the acetylated second mixture with a purine or pyrimidine base or analogue or derivative thereof and a Lewis Acid to produce a dioxolane nucleoside analogue.

DETAILED DESCRIPTION OF THE INVENTION

The present invention involves a high yield process of separating β and α anomers from an anomeric mixture of dioxolane nucleoside analogue precursors which provides higher yield and greater efficiency. In one embodiment, this method is used in the production of dioxolane nucleoside analogues having a high degree of anomeric purity at lower cost. Additionally, another aspect of the present invention involves synthesizing starting material having a higher degree of anomeric purity.

The present invention provides a process of preparing dioxolane nucleoside analogues having a predominant β-L-configuration using enzymes, namely hydrolases. The procedure improves overall yield and has relatively few steps, thereby improving overall efficiency. The process involves the following steps.

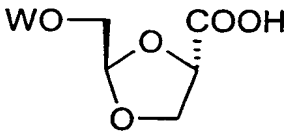
A mixture of anomers represented by formula A or formula B is obtained as described herein in Scheme 1.



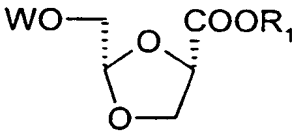
(A) (B)

In the above formula, W is benzyl or benzoyl, and R_1 is selected from the group consisting of H, C_{1-6} alkyl and C_{6-15} aryl. The mixture is hydrolyzed with an enzyme selected from the group consisting of cholesterol esterase, ESL-001-02, horse liver esterase, bovine

pancreatic protease, α-chymotrypsin, protease from *Streptomyces caespitosis*, substilisin from *Bacillus licheniformis*, protease from *Aspergillus oryzae*, proteinase from *Bacillus licheniformis*, protease *from Streptomyces griseus*, acylase from *Aspergillus melleus*, proteinase from *Bacillus subtilis*, ESL-001-05, pronase protease from *Streptomyces griseus*, lipase from *Rhizopus arrhizus*, lipoprotein lipase from *Pseudomonas* species type B, lipase from *Pseudomonas cepacia* and bacterial proteinase. The hydrolyzing step stereoselectively hydrolyzes the α-anomer of the mixture of either formula A or formula B. The result is an unhydrolysed β-anomer. The α-anomer can be separated easily from the β-anomer. If an anomeric mixture of the compound of formula A is selected, the result is the production of the compound of formula C and formula D:

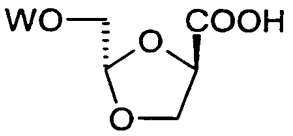


(C)

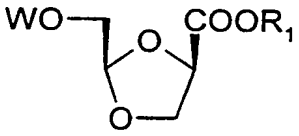


(D)

If an anomeric mixture of the compound of formula B is selected, the result is the production of the compound of formula E and formula F:



(E)



(F)

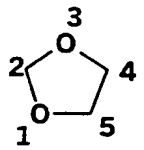
The mixture (C)/(D) or (E)/(F) is then subjected to oxidative decarboxylation which replaces the R_1 group with an acyl moiety. It is then glycosylated with a purine or pyrimidine base or analogue or derivative thereof in the

presence of a Lewis Acid. The final step produces a dioxolane nucleoside analogue in the β-L configuration for the mixture (C)/(D) and a dioxolane nucleoside analogue in the β-D configuration for the mixture (E)/(F).

At the outset, the following definitions have been provided as reference. Except as specifically stated otherwise, the definitions below shall determine the meaning throughout the specification.

"Nucleoside" is defined as any compound which consists of a purine or pyrimidine base, linked to a pentose sugar.

"Dioxolane nucleoside analogue" is defined as any compound containing a dioxolane ring as defined hereinafter linked to a purine or pyrimidine base or analogue or derivative thereof. A "dioxolane ring" is any substituted or unsubstituted five member monocyclic ring that has an oxygen in the 1 and 3 positions of the ring as illustrated below:



Dioxolane Ring

"Purine or pyrimidine base" is defined as the naturally occurring purine or pyrimidine bases adenine, guanine, cytosine, thymine and uracil. A purine or pyrimidine that is a moiety is a purinyl or pyrimidinyl, respectively.

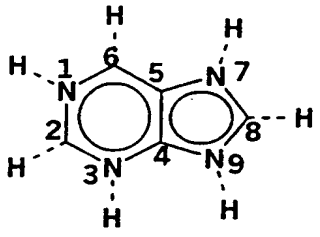
"Alkyl" is defined as a substituted or unsubstituted, saturated or unsaturated, straight chain, branched chain or carbocyclic moiety, wherein the straight chain,

branched chain or carbocyclic moiety can be optionally interrupted by one or more heteroatoms (such as oxygen, nitrogen or sulfur). A substituted alkyl is substituted with a halogen (F, Cl, Br, I), hydroxyl, amino or C_{6-20} aryl.

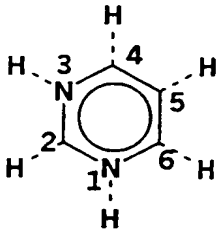
"Aryl" is defined as a carbocyclic moiety which can be optionally substituted or interrupted by one heteroatom (such as oxygen, nitrogen or sulfur) and containing at least one benzenoid-type ring (such as phenyl and naphthyl).

"Carbocyclic moiety" is defined as a substituted or unsubstituted, saturated or unsaturated, C_{3-6} cycloalkyl wherein a substituted cycloalkyl is substituted with a C_{1-6} alkyl, halogen (i.e. F. Cl, Br, I), amino, carbonyl (i.e. COOH), or NO_2.

A "derivative" of a purine or pyrimidine base refers to one of the following structures:



Purine



Pyrimidine

wherein one or more of the pyrimidine H are substituted with substituents that are known in the art. In the above illustration, the bonds represented by a broken line are optional and are present only in cases which require the bond to complete the valence of the ring atom. Substitutents bound to the ring members by a

single bond include but are not limited to halogen such as F, Cl, Br, I; an akyl such as lower akyls; aryl; cyano carbamoyl; amino including primary, secondary and tertiary amino; and hydroxyl groups. Substituents bound to the carbon ring atoms by a double bond include but are not limited to a =O to form a carbonyl moiety in the ring. It is understood that when the ring is aromatic, some of the substitutions may form tautomers. The definition shall include such tautomers.

"Analogue" of a purine or pyrimidine base refers to any derivative of purine or pyrimidine bases that is further modified by substituting one or more carbon in the ring structure with a nitrogen.

"Stereoselective enzymes" are defined as enzymes which participate as catalysts in reactions that selectively yield one specific stereoisomer over other stereoisomers.

"Anomeric purity" is defined as the amount of a particular anomer of a compound divided by the total amount of all anomers of that compound present in the mixture multiplied by 100%.

"Alkoxy" is defined as an alkyl group, wherein the alkyl group is covalently bonded to an adjacent element through an oxygen atom (such as methoxy and ethoxy).

"Alkoxycarbonyl", is defined as an alkoxy group attached to the adjacent group of a carbonyl.

"Acyl" is defined as a radical derived from a carboxylic acid, substituted (by a halogen, C_{6-20} aryl or C_{1-6} alkyl) or unsubstituted by replacement of the -OH group. Like

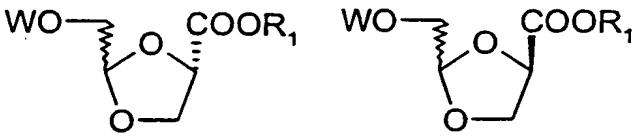
the acid to which it is related, an acyl radical may be aliphatic or aromatic, substituted (by halogen, C_{1-6} alkoxyalkyl, nitro or O_2) or unsubstituted, and whatever the structure of the rest of the molecule may be, the properties of the functional group remain essentially the same (such as acetyl, propionyl, isobutanoyl, pivaloyl, hexanoyl, trifluoroacetyl, chloroacetyl and cyclohexanoyl).

"Alkoxyalkyl" is defined as an alkoxy group attached to the adjacent group by an alkyl group (such as methoxymethyl).

"Acyloxy" is defined as an acyl group attached to the adjacent group by an oxygen atom.

"Oxo" is defined as a =O substituent bonded to a carbon atom.

As noted above, one embodiment of the present invention is a process for separating β and α anomers from an anomeric mixture represented by the following formula A or formula B:



(A) (B)

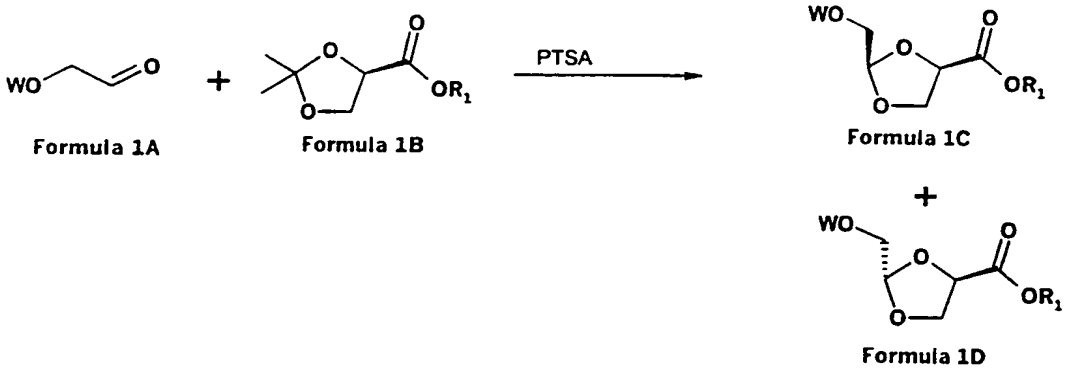
wherein W is benzyl or benzoyl and R_1 is selected from the group consisting of C_{1-6} alkyl and C_{6-15} aryl.

In one embodiment, the process stereoselectively hydrolyses predominantly the α-anomer to form a product

where R_1 of formula A and formula B is replaced with H. The β-anomer remains substantially unhydrolyzed. The process also comprises separating the hydrolyzed product from unhydrolyzed starting material.

The process of making a β-L dioxolane nucleoside analogue begins with the preparation of starting materials. Scheme 1 depicts the manufacture of a mixture that includes formula A or B.

Scheme 1



An oxyacetaldehyde represented by formula 1A (wherein W is benzyl or benzoyl) is reacted with 1,3-dioxolane-4-(4R)carboxylic acid-2,2-dimethyl-methyl ester (formula 1B) in approximately equimolar proportions. The dioxolane of formula 1B has a chiral center at the C4 carbon. The reaction occurs in a toluene solvent. The mixture is heated to 58°C. The catalyst, PTSA, is added. The mixture is heated to a temperature between 64-67°C. A vacuum is applied at 70 kPa, and the reaction proceeds for 40 minutes. Traces of solvent are then removed by high vacuum. The catalyst is removed by filtration using a 1:1 ratio of Hexane:EtOAc as an eluent. In one embodiment, the preferred filter is a silica gel pad.

The resulting product is a crude oil containing a mixture of the compounds of formula 1C and 1D, wherein the ratio is 2:1 of (1C:1D) respectively.

It can be appreciated by a person of skill in the art that the reaction conditions can be adjusted to optimize the purity of the stereoisomers. In one embodiment of the present invention, the reaction of the compound of formula 1A with the compound of formula 1B is done in the presence of catalyst in an amount between about 1.0 wt% and 10.0 wt% of the starting material. In another embodiment the amount of catalyst is between about 2.5 wt% and about 5.5 wt% of the starting materials. In yet another embodiment, the amount of catalyst is between about 3.0 wt% and about 5.0 wt%. In still another embodiment, the amount of catalyst is between about 3.5%. and about 5.5%. In another embodiment, the amount of catalyst is between about 2.5 wt% and about 7.5 wt%. In another embodiment the amount of catalyst is about 5.0 wt%.

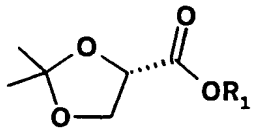
In an embodiment of the present invention, the reaction of the compound of formula 1A with the compound of formula 1B is done at a temperature ranging from about 40°C to about 80°C. In another embodiment of the present invention, the temperature ranges from about 50°C to about 75°C. In still another embodiment, the temperature ranges from about 60°C to about 70°C. In an additional embodiment, the temperature ranges from about 65°C to about 79°C.

In an embodiment of the present invention, the reaction time between the compound of formula 1A and the compound of formula 1B corresponds to a period ranging from about

30 minutes to about 2 hours. In yet another embodiment, the period ranges from about 30 minutes to about 1 hour. In still another embodiment, the period ranges from about 30 minutes to about 50 minutes.

It will be appreciated by a person of ordinary skill in the art that the C4 carbon is chiral. Because this carbon is not involved in the reaction, the chirality is preserved at that carbon. A starting material can be selected to have a (4S) or (4R) stereochemistry.

According to one embodiment, it is preferable that the resulting product is an anomeric mixture favoring the β-L configuration over the α-L configuration. To achieve such a result, the starting material represented by formula 1B (4S) is selected and shown below:

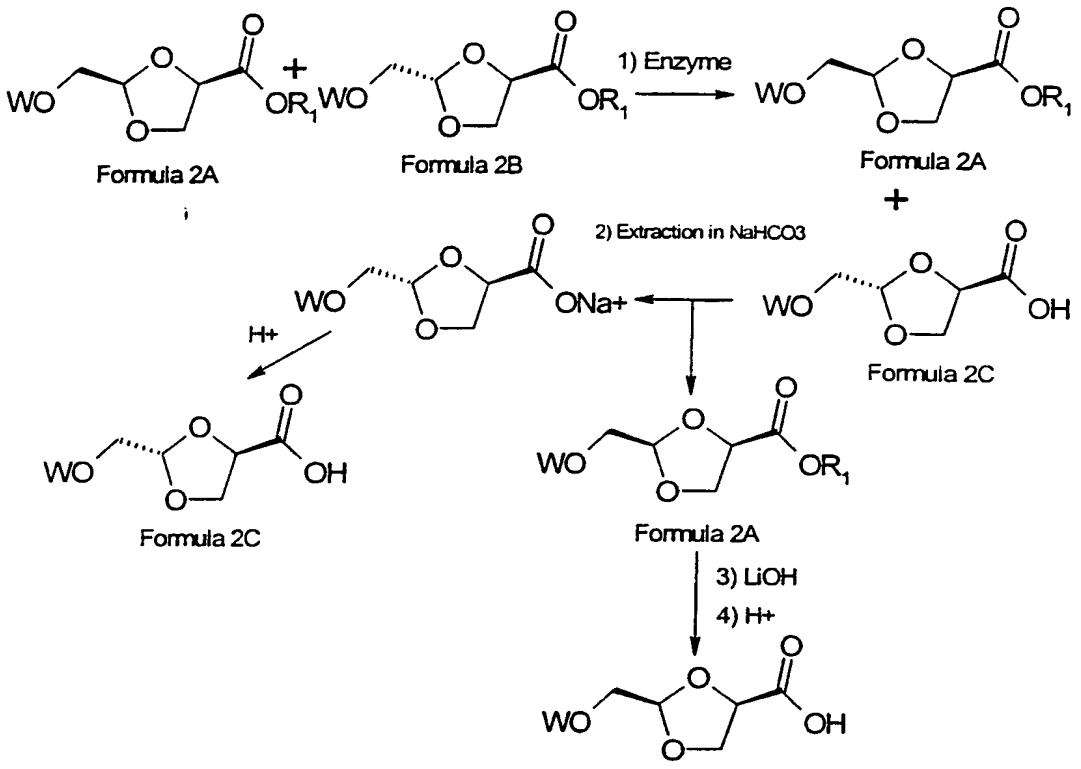


Formula 1B (4S)

The reaction proceeds according to the principles described above. The resulting product, according to one embodiment, will have an anomeric purity of the β-L anomer over the α-L anomer of greater than 55%, preferably 60% and more preferably 65%.

According to one embodiment, the present invention is a method of separating β-anomers from α-anomers according to the following Scheme 2:

Scheme 2



According to one embodiment, a mixture of anomers is obtained as represented by formula 2A or formula 2B. A mixture represented by formula 2A or formula 2B can be obtained according to the reaction described above or according to any method known in the art.

The reaction is prepared as follows: A portion of the material represented by formula 2A and formula 2B is weighed into a reaction vessel. For a small scale reaction, about 0.001 mmol of the mixture of formula 2A and formula 2B is added to about 46 mL of a 5 mmol solution of BES buffer (for a final concentration of

about 2 mM in substrate). For a preparative scale reaction, about 0.8 mmol of the mixture is added to about 10 mL of buffer (for a final concentration of about 80 mM in substrate). The pH of the buffer, according to one embodiment, should be between 7.0 and 7.5 and preferably 7.2.

The enzyme is selected from the group consisting of cholesterol esterase, ESL-001-02, horse liver esterase, bovine pancreatic protease, α-chymotrypsin, protease from *Streptomyces caespitosis*, substilisin from *Bacillus licheniformis*, protease from *Aspergillus oryzae*, proteinase from *Bacillus licheniformis*, protease from *Streptomyces griseus*, acylase from *Aspergillus melleus*, proteinase from *Bacillus subtilis*, ESL-001-5, pronase protease from *Streptomyces* griseus, lipase from *Rhizopus arrhizus*, lipoprotein lipase from *Pseudomonas* species type B, lipase from *Pseudomonas cepacia* and bacterial proteinase.

The commercial sources of the enzymes are readily available to a person of ordinary skill in the art. Particularly, some of the materials can be obtained from the following sources: Bovine cholesterol esterase was purchased from Genzyme (Cambridge, MA); ESL-001-02 from Diversa Corp. (San Diego, CA); Horse liver esterase and subtilisin from Bacillus licheniformis from Fluka Chemie (Oakville, ON); Bovine pancreas protease type 1, α-chymotrypsin and *Streptomyces caespitosis* from Sigma-Aldrich (Oakville, ON).

In another embodiment, the enzyme is selected from the group consisting of protease from *Aspergillus oryzae*,

proteinase from *Bacillus licheniformis*, subtilisin from *Bacillus licheniformis*, protease *from Streptomyces griseus*, acylase from *Aspergillus melleus*, proteinase from *Bacillus subtilis*, ESL-001-005, pronase protease from *Streptomyces griseus*, lipase from *Rhizopus arrhizus*, lipoprotein lipase from *Pseudomonas* species type B, bacterial proteinase and lipase from *Pseudomonas cepacia*. The selection of one of these enzymes is preferred according to this embodiment when the oxyacetaldehyde represented by the compound of formula 1A in Scheme 1 is selected to be benzoyloxyacetaldehyde.

According to another embodiment of the invention, the oxyacetaldehyde represented by the compound of formula 1A is benzoyloxyacetaldehyde. According to this embodiment, the enzyme is selected from the group consisting of protease from *Aspergillus oryzae*, proteinase from *Bacillus licheniformis*, subtilisin from *Bacillus licheniformis*, pronase protease from *Streptomyces griseus*, and lipase from *Rhizopus arrhizus*. In yet another embodiment, the enzyme is selected from the group consisting of protease from *Aspergillus oryzae* and proteinase from *Bacillus licheniformis*.

In yet another embodiment, the enzyme is selected from the group consisting of cholesterol esterase, ESL-001-02, horse liver esterase, bovine pancreatic protease, α-chymotrypsin, protease from *Streptomyces caespitosis* and substilisin from *Bacillus licheniformis*. The selection of one of these enzymes is preferred according to this embodiment when the oxyacetaldehyde represented by the compound of formula 1A is benzyloxyacetaldehyde.

The stereospecific enzyme selected is then added to begin

the hydrolysis reaction. The enzymatic reaction hydrolyzes primarily the α-anomer by replacing the R_1 group of the α-anomer of the compound of formula 2B with H to form the compound of formula 2C. The amount of the enzyme added can be determined according to principles known by any person of ordinary skill in the art. According to another embodiment, about 500 μL was added to begin the reaction. The rate and degree of hydrolysis was monitored by a pH-stat according to principles known in the art. As the compound of formula 2B is hydrolyzed, the pH of the mixture decreases. Thus, the change in pH as monitored by a pH stat corresponds to the completeness of the reaction.

If the reaction time is allowed to proceed longer than the optimal reaction time, the β-anomer may be converted resulting in lower anomeric purity of the final product. If the reaction time is too short, less than optimal amount of the α-anomer is converted resulting in a lower anomeric purity of the remaining unhydrolyzed reactant. According to one embodiment, the reaction is allowed to proceed until 43% completion. It will be appreciated by a person of ordinary skill in the art that the exact degree of completion may change depending upon the reactant used, the enzyme used and other principles known to a person of ordinary skill in the art.

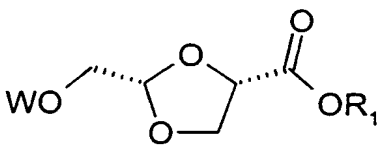
As noted, the ester starting material is separated from the hydrolyzed acid product when the desired endpoint is reached. The ester starting material and the hydrolysed product are separated by increasing the pH of the solution to more than pH 7.0 with bicarbonate solution and extracting with ethyl acetate (for example, 3 x 20

mL). The unhydrolyzed starting material is extracted in the ethyl acetate and the hydrolyzed product remains in salt form in the aqueous solution. The pH of the solution is then adjusted to pH 2. The hydrolyzed product is further extracted with ethyl acetate (for example, 3 x 20 mL). The reactants and the products are dried with $MgSO_4$, filtered and concentrated *in-vacuo*.

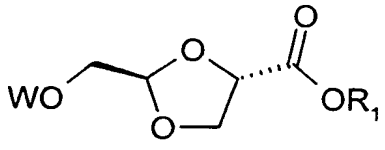
Alternatively, the unhydrolyzed product can be hydrolyzed by procedures known in the art such as reaction with LiOH followed by acidification.

Because of the enzyme selectivity, the anomeric purity of the hydrolyzed and separated β-anomer is considerable. Furthermore, the anomeric purity of the unhydrolyzed and separated α-anomer is also considerable. In one embodiment, the anomeric purity of the respective separated α-anomer and/or the β-anomer is greater than 80%. In another embodiment, the anomeric purity of the respective separated α-anomer and/or the β-anomer is greater than 90%. In another embodiment, the anomeric purity of the respective separated α-anomer and/or the β-anomer is greater than 95%. In another embodiment, the anomeric purity of the respective separated α-anomer and/or the β-anomer is greater than 98%.

In another embodiment, the procedure of Scheme 2 is followed except the anomeric mixture represented by formula 2A or 2B is replaced with an anomeric mixture represented by formula 2D and 2E, respectively.

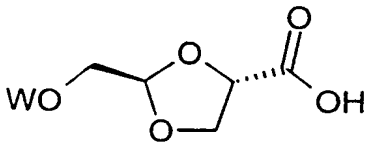


Formula 2D



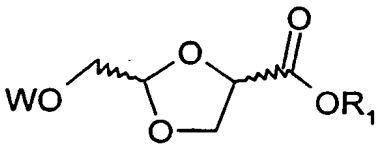
Formula 2E

According to this embodiment the α-anomer represented by formula 2E is hydrolyzed. The result is the separation of the hydrolyzed α-anomer represented by formula 2F from the unhydrolyzed β-anomer represented by formula 2D.



Formula 2F

In another embodiment, the procedure of Scheme 2 is followed except a mixture represented by formula 2A and 2B is replaced with a mixture of four stereoisomers represented by formula 2G.



Formula 2G

According to this embodiment, the α-anomer containing both D and L enantiomers is hydrolyzed. The result is the separation of the hydrolyzed α-anomer containing both D and L enantiomers from the unhydrolyzed β-anomer containing both D and L enantiomers.

After hydrolysis, purification and oxidative decarboxylation, the resulting dioxolane ring can be linked with a purine or pyrimidine base or analogue or derivative. There are several examples known by skilled artisan on how to link a purine or pyrimidine base or analogue or derivative to the dioxolane ring. For example, PCT Publ. No. WO/97/21706 by Mansour et al. describes one method of stereoselectively attaching the purine or pyrimidine base or analogue or derivative to a dioxolane ring. WO/97/21706 is incorporated herein fully by reference.

According to the process disclosed in WO/97/21706 the starting material is an acylated dioxolane ring. The starting material of the procedure disclosed in WO/97/21706 can be obtained by oxidative decarboxylation of a product of Scheme 2 discussed above. Oxidative decarboxylation destroys the stereochemistry of the C4 carbon while preserving the stereochemistry of the C2 carbon.

As noted, the oxidative decarboxylation step occurs after the hydrolysis step of Scheme 2. A compound having the desired stereochemistry on the C2 carbon is selected. For each mmol of compound that is processed, it is dissolved in between about 2.5 and about 4.0 mL of acetonitrile. In another embodiment, between about 3.0 and about 3.5 mL of acetonitrile was added for each mmol of compound. In yet another embodiment, between about 3.3 and about 3.4 mL of acetonitrile was added for each mmol of compound.

For each mmol of compound, between about 0.08 and about 0.12 mL of pyridine was added. In another embodiment,

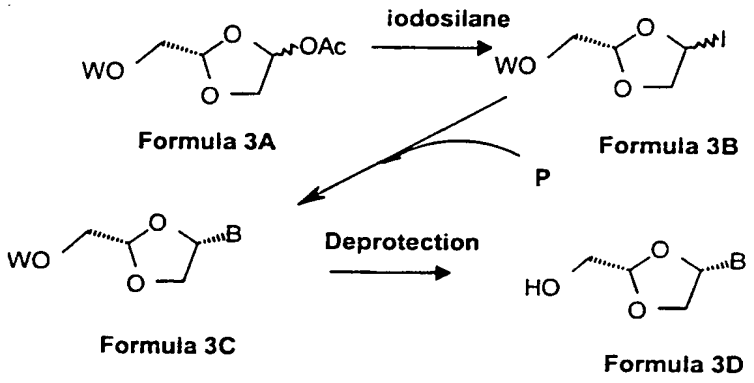
between about 0.09 and about 0.11 mL of pyridine was added for each mmol of compound. In yet another embodiment, approximately 0.1 mL of pyridine was added for each mmol of compound.

To this mixture, between 1.1 and 1.5 mmoles of $Pb(OAc)_4$ was added for each mmol of compound. In another embodiment, between about 1.2 mmoles and about 1.4 mmoles of $Pb(OAc)_4$ is added for each mmol of compound. In yet another embodiment, about 1.3 mmoles of $Pb(OAc)_4$ is added for each mmol of compound.

Thereafter, the mixture was stirred for 18 hours at room temperature. Then, the mixture was poured into a saturated solution of $NaHCO_3$. Between approximately 2.0 and 3.0 mL of $NaHCO_3$ were used for each mmol of compound. In one embodiment, between about 2.5 mL and about 2.7 mL, and more preferably about 2.6 mL of $NaHCO_3$ was used for each mmol of compound. The solution was then stirred for an additional 30 minutes. The organic layer was separated from the aqueous layer by four extractions of ethyl acetate. Extracts were combined, dried on anhydrous Na_2SO_4 and evaporated under a vacuum. Optionally, the crude can be further purified by chromatography on silica gel using a gradient of 0-15% ethyl acetate in hexane.

In one embodiment of the present invention, the oxidative decarboxylation step is followed by glycosylation. The glycosylation is represented by the following Scheme 3.

SCHEME 3



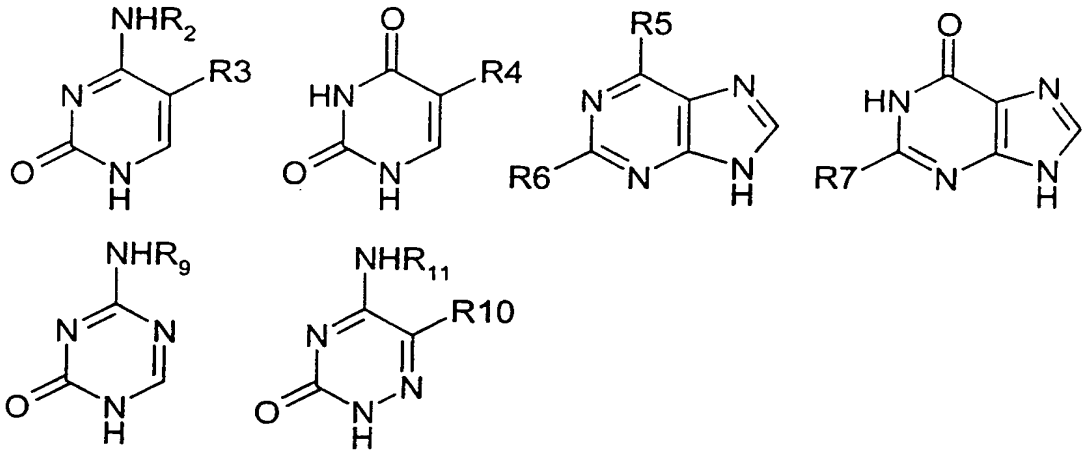
The first step in the glycosylation procedure is to obtain a compound with the desired stereospecificity at the C2 carbon. According to one embodiment, a compound having an S stereochemistry at the C2 carbon, as represented by the compound of formula 3A is preferred. The result is that a higher ratio of the β-L anomer is in the product 3C. According to another embodiment, compound having an R stereochemistry at the C2 carbon is preferred. The result is a product that has a higher ratio of the β-D anomer in the final product.

The compound of formula 3A is reacted with an iodosilane to produce the compound of formula 3B. In one embodiment, the iodosilane is iodotrimethylsilane.

In another embodiment, the iodosilane is diiodosilane. Important to the reaction is that it occurs at low temperatures. According to one embodiment, the temperature is preferably between 0 °C and -78°C prior to

glycosylation with silylated pyrimidine or purine base or analogue or derivative thereof.

The iodo intermediate represented by formula 3B is then dissolved in dichloromethane and is cooled down to between 0° C and -78° C. A purine or pyrimidine base or analogue or derivative thereof is then selected. According to one embodiment, the purine or pyrimidine base or analogue or derivative thereof is selected from the following group:



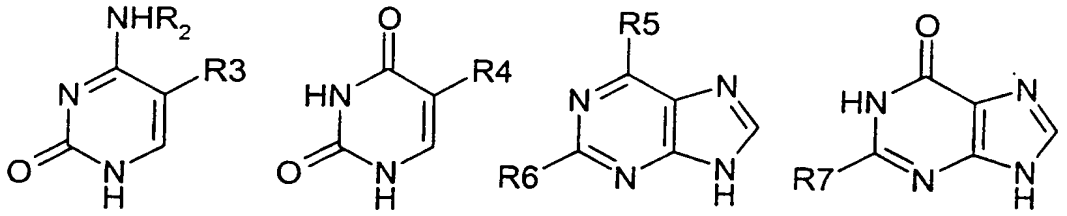
Wherein:

R_2, R_9 and R_{11} are each independently selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6} acyl and $R_8C(O)$ wherein R_8 is hydrogen or C_{1-6} alkyl;

R_3, R_4 and R_{10} are each independently selected from the group consisting of hydrogen, C_{1-6} alkyl, bromine, chlorine, fluorine, iodine and CF_3; and

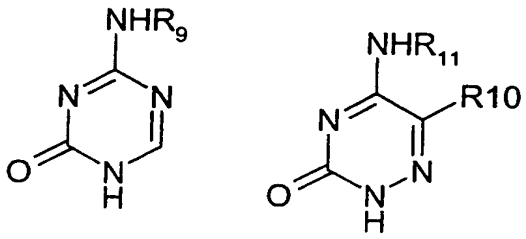
R_5, R_6 and R_7 are each independently selected from the group consisting of hydrogen, bromine, chlorine, fluorine, iodine, amino, hydroxyl and C_{3-6} cycloalkylamino.

According to one embodiment, the purine or pyrimidine base or derivative is selected from the group consisting of:



In this embodiment, R_2 is selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6} acyl and $R_8C(O)$ wherein R_8 is hydrogen or C_{1-6} alkyl. Additionally, R_3 and R_4 are each independently selected from the group consisting of hydrogen, C_{1-6} alkyl, bromine, chlorine, fluorine, iodine and CF_3; and R_5, R_6 and R_7 are each independently selected from the group consisting of hydrogen, bromine, chlorine, fluorine, iodine, amino, hydroxyl and C_{3-6} cycloalkylamino.

In another embodiment, the purine or pyrimidine base or analogue or derivative thereof is selected from the group consisting of:



In this embodiment, R_9 and R_{11} are independently selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6} acyl and $R_8C(O)$. Additionally, R_{10} is selected from the

group consisting of hydrogen, C_{1-6} alkyl, bromine, chlorine, fluorine, iodine and CF_3.

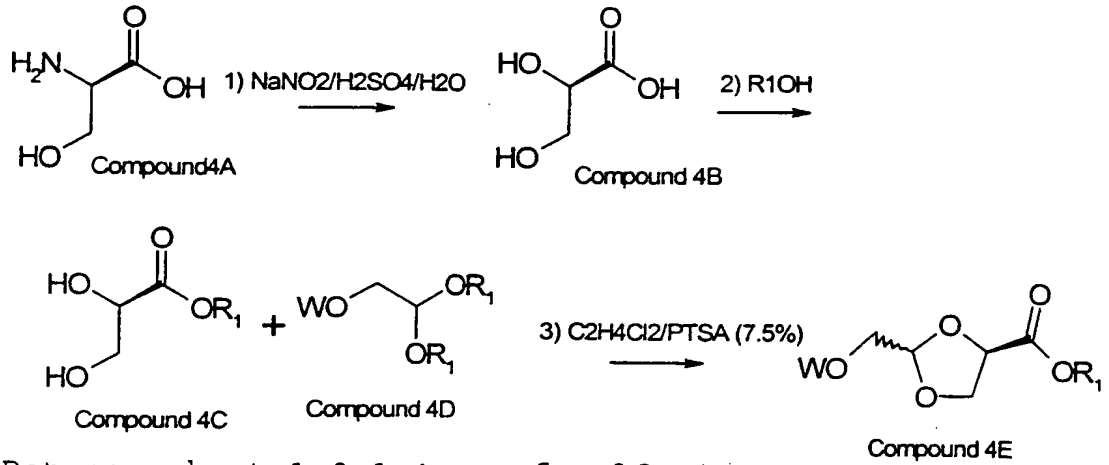
The purine or pyrimidine or analogue or derivative thereof is persylated by a sylating agent and ammonium sulphate followed by evaporation of HMDS to form a persylated purine or pyrimidine base or analogue or derivative thereof herein referred to as the persylated base and designated as P in Scheme 3. According to one embodiment, the sylating agent is selected from the group consisting of 1,1,1,3,3,3-hexamethyldisilazane, trimethylsilyl triflate, t-butyldimethylsilyl triflate or trimethylsilyl chloride. In one embodiment, the sylating agent is 1,1,1,3,3,3,-hexamethyldisilazane.

The persylated base P was dissolved in 30 mL of dichloromethane and was added to the iodo intermediate represented by formula 3B. The reaction mixture was maintained at between 0 and -78°C for 1.5 hours then poured onto aqueous sodium bicarbonate and extracted with dichloromethane (2x25 mL). The organic phase was dried over sodium sulphate to obtain the compound of formula 3C. As used in Scheme 3, the B represents a moiety of the purine or pyrimidine base or analogue or derivative thereof which was persylated in the above step to form P. The compound of formula 3C was removed by filtration and the solvent was evaporated *in-vacuo* to produce a crude mixture. The product represented by formula 3C has predominantly a 4S configuration at the C4 carbon with an anomeric purity of 80%. When the starting material is a compound represented by formula 3A, the product forms predominantly the β-L enantiomer having an anomeric purity of 80%.

Next, the compound of formula 3C is deprotected to produce the compound of formula 3D. This can be accomplished by dissolving a compound represented by formula 3C in ethanol and then adding cyclohexene and palladium oxide. The deprotection step can also be done by other methods which are well known by those skilled in the art. The reaction mixture is refluxed for 7 hours. It is then cooled and filtered to remove solids. The solvents are removed from the filtrate by vacuum distillation. The product represented by formula 3D is purified by flash chromatography on silica-gel (5% MEOH in ethylacetate). The deprotection step can also be done by other methods that are well known by a person skilled in the art.

In another embodiment, compounds of Scheme 1 may be prepared by an alternative process which is shown below in Scheme 4.

Scheme 4



Between about 1.0-1.4 eq of sulfuric acid was added in portions to a large excess of water while stirred at a temperature between 0-5°C. By way of example and not by limitation, if 9.06 mol of D-Serine represents 1

equivalent of reactant, then between about 9.5-13.3 mol of sulfuric acid is added to 7.3 L of water. In another embodiment, between about 1.1-1.3 eq of sulfuric acid was added to an excess of water. In a further embodiment, 1.2 eq of sulfuric acid was added to an excess of water.

About 1 equivalent of D-Serine was added in one portion under vigorous stirring. Then, between about 1.0 and 1.4 eq. of aqueous sodium nitrite was added dropwise. The temperature was kept between 0-5°C during the addition time (about seven hours). The reaction vessel was stirred overnight at room temperature. The water was removed by vacuum and the residue (D-glyceric acid) co-evaporated with toluene (3X1L). The residue was then stirred with about 6L of an alcohol solvent for about 30 minutes. According to one embodiment, the alcohol is of the formula R_1OH wherein R_1 is a C_{1-4} alkyl. According to another embodiment, the alcohol is methanol or ethanol. The resulting solid was removed by filtration. The clear solution was stirred at room temperature for 30-40 hours, the alcohol removed by vacuum to yield a D-glycerate in the form of a yellow viscous syrup. The D-glycerate is then reacted with between about 0.9-1.1 eq of a dialkyl acetal at a temperature of about 85-95°C. Examples of suitable dialkyl acetals include benzoyloxyacetaldehyde dialkyl acetal and benzyloxyacetaldehyde dialkyl acetal. Examples of suitable alkyls for the dialkyl acetal is methyl and ethyl.

Then, between about 1 wt% and about 10 wt% of PTSA is added. According to another embodiment, about 5 wt% PTSA is added. In another embodiment, about 0.02 eq. of solid PTSA is added. The reaction mixture is kept under vacuum at a temperature between 85-95°C for 2-3 hours. The

mixture is then cooled to room temperature, diluted with ethylacetate (250 mL) and poured onto saturated sodium bicarbonate solution (250 mL) under stirring. The organic phase is separated and the aqueous phase concentrated, purified on a silica gel column eluting with 5-10% ethylacetate/hexanes to yield the desired dioxolane as a light yellow oil (about 59%) with β/α ratio of 2:1 or higher.

Alternatively, the reactants of step 3 of Scheme 4 can be substituted by corresponding reactants of Scheme 1. For example, the D-glycerate represented by Formula 4C is replaced with an 1,3 dioxolane-4-(4R)carboxylic acid-2,2-dimethyl alkyl ester represented by Formula 1B according to one embodiment. Additionally or alternatively, the dialkyl acetal represented by Formula 4D is replaced with an oxyaldehyde represented by Formula 1A. These substitutions do not require changing the reaction conditions substantially disclosed above for the third step of Scheme 4.

In a further embodiment of the present invention, the starting material of Scheme 4 is L-Serine which produces an end product having an S-configuration at the C4 carbon of the dioxolane ring. Alternatively, the L-glycerate of Step 3 can be replaced with an 1,3 dioxolane-4-(4S)carboxylic acid-2,2-dimethyl alkyl ester to produce an end product having predominantly an S-configuration at the C4 carbon of the resulting dioxolane ring.

Example 1. Enzyme catalyzed hydrolytic resolution of the dioxolane methyl ester.

A 2:1 (β:α) anomeric mixture of (2-(S)-benzyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester) (136.5 mg, 0.541mmol) was weighed into a reaction vessel and BES buffer(6,263 mL of a 5 mM solution, pH 7.2) was added. The substrate remained as insoluble droplets. α-Chymotrypsin (500 µL of a 5mg/mL BES buffer, pH 7.2 solution, 0.019 units by 4-nitrophenylacetate assay) was added to begin the reaction and the rate and degree of hydrolysis was monitored by a pH-stat which maintained the pH at 7 by automatic titration with 0.0981 mmol NaOH. The reaction was terminated at 43% conversion for high anomeric purity as determined by Sih's equations for recycling (Chen. C.S.; Fujimoto, Y.; Girdaukas, G.; Sih, C.J., J.AM. Chem. Soc. 1982, 104, 7294-99), by extracting the remaining starting material ester with ethyl acetate (3x20mL). The aqueous layer was adjusted to pH 2 and the product acid extracted with ethyl acetate (3x20mL).

Both extracts were dried with $MgSO_4$, filtered and concentrated *in-vacuo*. By this method, we obtained the (2-(S)-benzyloxymethyl)-4-(S)-carboxylic acid-1,3-dioxolane methyl ester).

Example 2. Purity of β-Anomer by NMR.

Analysis was performed on a Varian Gemini 200 MHz NMR spectrometer in $CDCl_3$. The α-ester shows a triplet at δ 5.33 (^3J = 4.6 Hz) and the β-ester shows a triplet upfield at δ 5.23 (^3J = 4. 6 Hz). The α-acid shows a triplet at δ 5.33(^3J = 3.6 Hz), while the β-acid shows a broad

singlet upfield at δ 5.19. We did not observe any epimerization of the substrate or product acid during work-up. By NMR analysis, the purity of the β-anomer is determined to have about 95% anomeric purity or higher.

Example 3: Purity of the α-Anomer.

The product acid is obtained from example 1 after it was dried with $MgSO_4$, filtered and concentrated *in-vacuo*. It is analyzed for purity by NMR. The α-anomer is isolated with high anomeric purity.

Example 4: Enzymatic Resolution of β-anomer with Protease from *Aspergillus oryzae*.

The procedure of Examples 1-2 were followed using protease from Aspergillus oryzae as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 5: Enzymatic Resolution of α-anomer with Protease from *Aspergillus oryzae*.

The product acid is obtained from Example 4 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 6: Enzymatic Resolution of β-anomer with Proteinase from *Bacillus licheniformis*.

The procedure of Examples 1-2 were followed using proteinase from bacillus licheniformis as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxalane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 7: Enzymatic Resolution of α-anomer with Proteinase from *Bacillus licheniformis*.

The product acid is obtained from Example 6 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 8: Enzymatic Resolution of β-anomer with Subtilisin from *Bacillus licheniformis*.

The procedure of Examples 1-2 were followed using subtilisin from bacillus licheniformis as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 9: Enzymatic Resolution of α-anomer with Subtilisin from *Bacillus licheniformis*.

The product acid is obtained from Example 8 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 10: Enzymatic Resolution of β-anomer with Protease from *Streptomyces griseus*.

The procedure of Examples 1-2 were followed using protease from streptomyces griseus as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 11: Enzymatic Resolution of α-anomer with Protease from *Streptomyces griseus*.

The product acid is obtained from Example 10 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 12: Enzymatic Resolution of β-anomer with Acylase from *Aspergillus melleus*.

The procedure of Examples 1-2 were followed using acylase from aspergillus melleus as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 13: Enzymatic Resolution of α-anomer with Acylase from *Aspergillus melleus*.

The product acid is obtained from Example 12 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 14: Enzymatic Resolution of α-anomer with Proteinase from *Bacillus subtilis*.

The procedure of Examples 1-2 were followed using proteinase from bacillus subtilis as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 15: Enzymatic Resolution of α-anomer with Proteinase from *Bacillus subtilis*.

The product acid is obtained from Example 14 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 16: Enzymatic Resolution of β-anomer with ESL-001-05

The procedure of Examples 1-2 were followed using diversa clonezymes #5 as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic

acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 17: Enzymatic Resolution of α-anomer with ESL-001-05

The product acid is obtained from Example 16 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 18: Enzymatic Resolution of β-anomer with Pronase protease from *Streptomyces griseus*.

The procedure of Examples 1-2 were followed using pronase from streptomyces as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 19: Enzymatic Resolution of α-anomer with Pronase protease from *Streptomyces griseus*.

The product acid is obtained from Example 18 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 20: Enzymatic Resolution of β-anomer with Lipase from *Rhizopus arrhizus*.

The procedure of Examples 1-2 were followed using Lipase from rhizopus arrhizus as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-

carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 21: Enzymatic Resolution of α-anomer with Lipase from *Rhizopus arrhizus*.

The product acid is obtained from Example 20 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 22: Enzymatic Resolution of β-anomer with Lipoprotein Lipase from Pseudomonas Species Type B.

The procedure of Examples 1-2 were followed using lipoprotein lipase from pseudomonas sp. type B as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high purity.

Example 23: Enzymatic Resolution of α-anomer with Lipoprotein lipase from Pseudomonas Species Type B.

The product acid is obtained from Example 22 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 24: Enzymatic Resolution of β-anomer with Bacterial Proteinase.

The procedure of Examples 1-2 were followed using bacterial proteinase as the enzyme to separate a 2:1

(β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 25: Enzymatic Resolution of α-anomer with Bacterial Proteinase.

The product acid is obtained from Example 24 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 26: Enzymatic Resolution of β-anomer with Lipase from *Pseudomonas cepacia*.

The procedure of Examples 1-2 were followed using lipase from pseudomonas cepacia as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-diaxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 27: Enzymatic Resolution of α-anomer with Lipase from *Pseudomonas cepacia*.

The product acid is obtained from Example 26 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 28: Enzymatic Resolution of β-anomer with Cholesterol esterase.

The procedure of Examples 1-2 were followed using cholesterol esterase as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzyloxymethyl)-4-

carboxylic acid-1,3-diaxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 29: Enzymatic Resolution of α-anomer with Cholesterol esterase.

The product acid is obtained from Example 28 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 30: Enzymatic Resolution of β-anomer with ESL-001-02.

The procedure of Examples 1-2 were followed using ESL-001-02 as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 31: Enzymatic Resolution of α-anomer with ESL-001-02.

The product acid is obtained from Example 30 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 32: Enzymatic Resolution of β-anomer with Horse Liver Esterase.

The procedure of Examples 1-2 were followed using horse liver esterase as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzyloxymethyl)-4-carboxylic

acid-1,3-diaxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 33: Enzymatic Resolution of α-anomer with Horse Liver Esterase.

The product acid is obtained from Example 32 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 34: Enzymatic Resolution of β-anomer with Bovine Pancreatic Protease.

The procedure of Examples 1-2 were followed using bovine pancreatic protease as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 35: Enzymatic Resolution of α-anomer with Bovine Pancreatic Protease.

The product acid is obtained from Example 34 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 36: Enzymatic Resolution of β-anomer Protease from *Streptomyces caespitosus*.

The procedure of Examples 1-2 were followed using protease from streptomyces caespitosis as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-

benzyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high anomeric purity.

Example 37: Enzymatic Resolution of α-anomer with Protease from *Streptomyces caespitosus*.

The product acid is obtained from Example 36 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

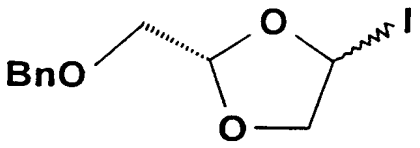
Example 38: Enzymatic Resolution of β-anomer with Substilisin from *Bacillus licheniformis*.

The procedure of Examples 1-2 were followed using substilisin from bacillus licheniformis as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzyloxymethyl)-4-carboxylic acid-1,3-dioxolane methylester). The result is a β-anomer that has high anomeric purity.

Example 39: Enzymatic Resolution of α-anomer with Substilisin from *Bacillus licheniformis*.

The product acid is obtained from Example 38 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high anomeric purity.

Example 40: Preparation of 2-(S)-Benzyloxymethyl-4-(R)-iodo-1,3-dioxolane and 2-(S)-Benzyloxymethyl-4-(S)-iodo-1,3-dioxolane (Compound 40).

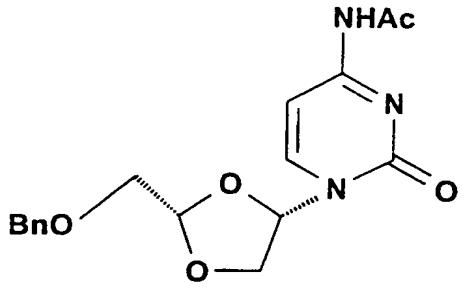


Compound 40

A mixture consisting of 2S-benzyloxymethyl-4S acetoxy-1,3-dioxolane and 2S-benzyloxymethyl-4R-acetoxy-1,3-dioxolane in 1:2 ratio (6g; 23.8 mmol) was dried by azeotropic distillation with toluene *in-vacuo*. After removal of toluene, the residual oil was dissolved in dry dichloromethane (60 mL) and iodotrimethylsilane (3.55 mL; 1.05 eq.) was added at -78°C, under vigorous stirring. The dry-ice/acetone bath was removed after addition and the mixture was allowed to warm up to room temperature (15 min.). The product was 2S-benzyloxymethyl-4R-iodo-1,3-dioxolane and 2S-benzyloxymethyl-4S-iodo-1,3-dioxolane.

It would be understood by a person of ordinary skill in the art that if the starting mixture was chosen consisting of 2R-benzyloxymethyl-4S acetoxy-1,3-dioxolane and 2R-benzyloxymethyl-4R-acetoxy-1,3-dioxolane. The resulting product is 2R-benzyloxymethyl-4R-iodo-1,3-dioxolane and 2R-benzyloxymethyl-4S-iodo-1,3-dioxolane. Furthermore, the starting material having a benzoyl substituent group instead of a benzyl would result in a product having a benzoyl substituent and not a benzyl.

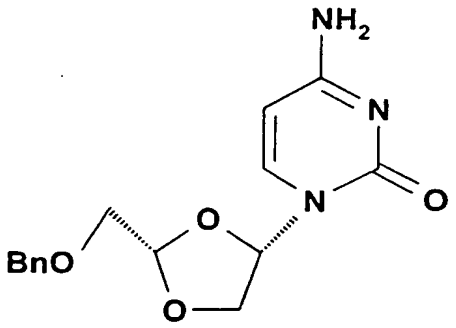
Example 41: Synthesis of 2-(S)-Benzyloxymethyl-1,3-dioxolan-4-(S)-yl)-2-oxo-4-aminoacetyl-pyrimidine (Compound 41).



Compound 41

The previously prepared iodo intermediate (Compound 40) in dichloromethane, was cooled down to -78° C. Persylated N-acetyl cytosine (1.1 eq) formed by reflux in 1,1,1,3,3,3-hexamethyl disilazane (HMDS) and ammonium sulphate followed by evaporation of HMDS was dissolved in 30 mL of dichloromethane and was added to the iodo intermediate. The reaction mixture was maintained at -78°C for 1.5 hours then poured onto aqueous sodium bicarbonate and extracted with dichloromethane (2x25mL). The organic phase was dried over sodium sulphate, the solid was removed by filtration and the solvent was evaporated *in-vacuo* to produce 8.1 g of a crude mixture. β-L-4'-benzyl-2'-deoxy-3'-oxacytidine and its α-L isomer were formed in a ratio of 5:1 respectively. This crude mixture was separated by chromatography on silica-gel (5% methanol in ethylacetate) to generate the pure β-L (β) isomer (4.48 g). Alternatively, recrystallization of the mixture from ethanol produces 4.92 g of pure β isomer and 3.18 g of a mixture of β and α-isomers in a ratio of 1:1.

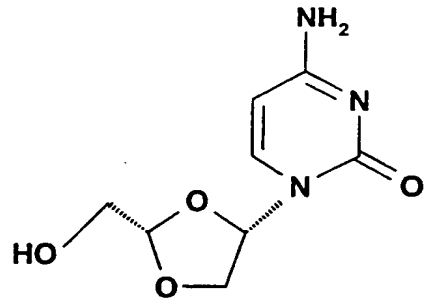
Example 42: 2-(S)-Benzyloxymethyl-1,3-dioxolan-4-(S)-yl)-2-oxo-4-amino-pyrimidine (Compound 42).



Compound 42

The protected β-L isomer (4.4 g) (Compound 41) was suspended in saturated methanolic ammonia (250 mL) and stirred at room temperature for 18 hours in a closed vessel. The solvents were then removed *in-vacuo* to afford the deacetylated nucleoside in pure form.

Example 43: 2-(S)-hydroxymethyl-1,3-dioxolan-4-(S)-yl)-2-oxo-4-amino-pyrimidine (Compound 43).



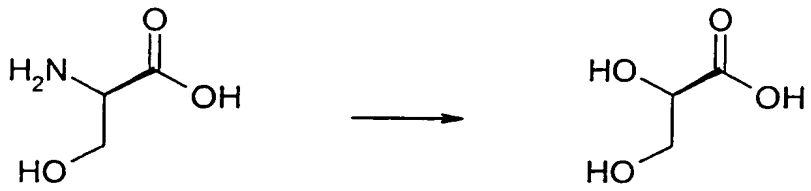
Compound 43

β-L-4'-Benzyl-2'-deoxy-3'-oxacytidine (Compound 42) was dissolved in EtOH (200 mL) followed by addition of cyclohexene (6 mL) and palladium oxide (0.8 g). The reaction mixture was refluxed for 7 hours then it was cooled and filtered to remove solids. The solvents were removed from the filtrate by vacuum distillation. The

crude product was purified by flash chromatography on silica-gel (5% MeOH in EtOAc) to yield a white solid (2.33 g; 86% overall yield). α_D^{22} = -46.7° (c = 0.285; MeOH) m.p. = 192 - 194°C.

The following examples 44-46 illustrate a method of preparing the starting material of example 1 (2-(S)-benzyloxymethyl-4-carboxylic acid-1,3-dioxolane methyl ester).

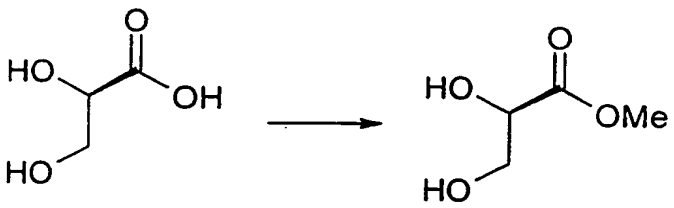
Example 44: Preparation of D-glyceric acid.



Portions of sulfuric acid (297 mL; 11.14 mol; 1.23 eq) was added to a large excess of water (7,300 mL) under stirring and cooling (0-5°C). D-Serine (952 g;9.06 mol;1 eq) was added in one portion under vigorous stirring, followed by dropwise addition of aqueous sodium nitrite (769 g;11.14 mol; 1.23 eq in 3,060 mL water). Temperature was kept between 0-5°C during the addition time (seven hours). The reaction vessel was stirred overnight at room temperature and the reaction monitored by TLC (ninhydrin). In order to complete the reaction, additional sulfuric acid (115 mL; 4.31 mol; 0.47 eq) and aqueous sodium nitrite (255 g; 3.69 mol; 0.4 eq in 1,100 mL water) was added, keeping the reaction vessel temperature between 0-5°C. The reaction vessel was then kept under stirring at room temperature for another 18 hours. Nitrogen was bubbled through the solution for one hour and the water removed by vacuum, keeping the

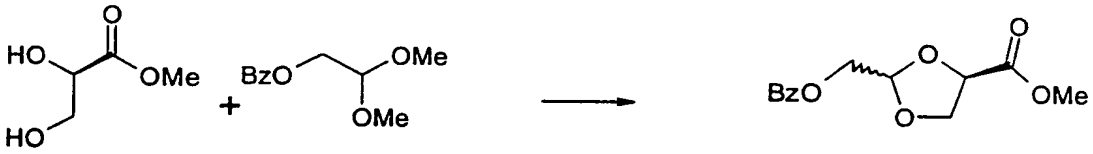
reaction vessel temperature between 28-30°C. The residue (D-glyceric acid) was co-evaporated with toluene (3X1L).

Example 45: Preparation of D-methyl glycerate.



D-glyceric acid was stirred with methanol (6L) for 30 minutes and the solid removed by filtration. The clear solution was stirred at room temperature for 35-38 hours and the reaction monitored by TLC (DCM/MeOH 8:2 Rf=0.63). Methanol was removed by vacuum to yield a yellow viscous syrup (1,100 g).

Example 46: Preparation of 2-(R,S)-benzoyloxymethyl-4-R-methylcarboxylate-1,3-dioxolane.

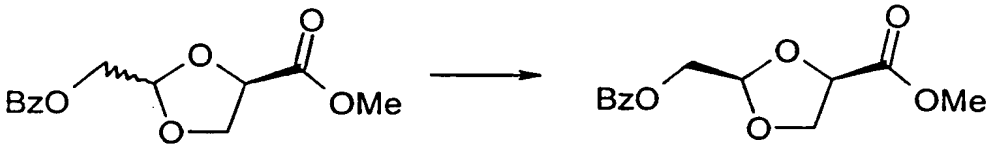


A mixture of benzoyloxyacetaldehyde dimethyl acetal (146 g, 95%, 0.66 mole, 1 eq) and D-methyl glycerate (99 g, 0.82 mole, 1.25 eq) was heated to 90oC, followed by the addition of solid PTSA (2.75 g, 0.145 moles, 0.022 eq). The reaction mixture was kept under vacuum (water aspirator) at 90-95°C for 2.5 hours (TLC, Hexanes/Ethylacetate 1:1, Rf-0.47). The reaction mixture was cooled down to room temperature, diluted with ethylacetate (250 mL) and poured onto saturated NaHCO3 solution (250 mL) under stirring. The organic phase was

separated and the aqueous phase was extracted one with ethylacetate (150 mL). The combined organic phase was concentrated and purified on a silica gel column eluting with 5-10% ethylacetate/hexanes to yield 112.4 g of the desired product as a light yellow oil (59%) with β/α ratio of 2.1:1.

The following example 47 illustrates a large scale version of the enzymatic separation of Example 1.

Example 47: Large scale preparation of β 2-(R)-benzoyloxymethyl-1,3-dioxolane-4-(R)-methylcarboxylate.



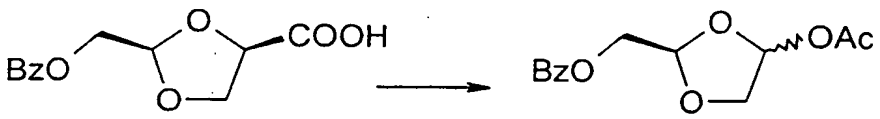
A mixture of β/α 2-benzoyloxymethyl-4-methylcarboxylate-1,3-dioxolane (20 g; 75.12 mmol) was suspended in acetonitrile (40 mL) and phosphate buffer (160 mL) at 30°C. α-Chymotrypsin was added (7.5 mL) in one portion followed by dropwise addition of a 1N solution of NaOH (total volume 46 mL) over six hours to hydrolyze the α isomer. The pH was constantly monitored and maintained between 7.1 and 7.2 while the temperature was kept at 30°C. After six hours, the mixture was extracted with EtOAc (1X80 mL). Then, the organic layer was separated, and the aqueous layer was extracted with EtOAc (2X50mL). Combined organic layers were washed with saturated $NaHCO_3$ (20 mL), dried over anhydrous Na_2SO_4 and the solvent removed under vacuum to leave 12.63 g of a clear oil (85.6%; β-isomer with less than 2.24% of the α-isomer by HPLC).

It is understood by a person of ordinary skill in the art that this example can be followed substituting chymotrypsin with any of the enzymes used in examples 4 through 39 to result in a large scale production of the β-isomer of 2-benzoyloxymethyl-4-methylcarboxylate-1,3-dioxolane.

Example 48. Preparation of β 2-(R)-benzoyloxymethyl-1,3-dioxolane-4-(R)-carboxylic acid.

β 2-(R)-benzoyloxymethyl-1,3-dioxolane-4-(R)-methylcarboxylate-1,3-dioxolane (15.327 g; 57.57 mmol) is dissolved in THF (60 mL) then water (15 mL) was added under stirring. The internal temperature was set to 20°C. Then, a solution of LiOH (2.41 g; 57.57 mmol) in water (15 mL) was added dropwise over 7 minutes. The reaction mixture was stirred at 22°C for an additional 40 minutes. THF was removed under vacuum, and the residue diluted with water (70 mL). The resulting solution was extracted with dichloromethane (2X35mL). The aqueous phase was acidified by 30% H_2SO_4 (9.5 mL) under tight pH-meter control (initial pH:8.36 to 3.02) then extracted with DCM (4X60mL). The organic phases were combined and the solvent removed under vacuum to furnish a light green syrup (14.26 g) which was kept under vacuum overnight.

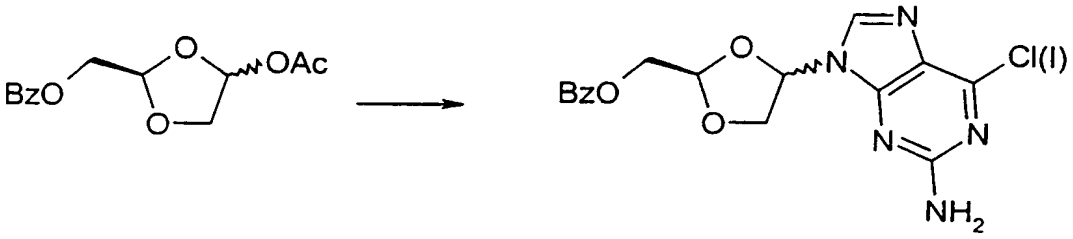
Example 49: Preparation of β 2-(R)-benzoyloxymethyl-4-(R,S)-methylcarboxylate-1,3-dioxolane.



Lead tetraacetate (944, 8 g; 2,024 mole; 1,2 eq) was added portion-wise to an acetonitrile (6.8 L) solution of the acid (425,5 g; 1,687 mole; 1,0 eq) and pyridine (193 mL) in an ice bath. The reaction vessel was allowed to warm up to room temperature and stirred. The reaction was checked by TLC (hexanes:ethyl acetate 6:4). It was filtered through a small pad of celite (about 1 inch). Then, the filtrate was poured onto 5 L of saturated aqueous sodium bicarbonate solution (reaction mixture turned brown), and the pH as adjusted to 8 by adding solid sodium bicarbonate. The filtrate was again filtered through a small pad of celite (about 1 inch) to remove the black lead salts to yield a pale yellow mixture. The organic phase was separated and the aqueous phase was extracted with ethylacetate (4X2L). The combined organic phase was concentrated, and the oil obtained was co-evaporated with toluene (3X2L) to yield a brown syrup.

This syrup (374 g) was further purified by filtering through a small pad of silica gel (1 g crude; 2 g silica), eluting with 3.5 L of the solvent mixture (ethyl acetate:hexanes 8:2) to yield 332,3 g (74%) of pure product. This last filtration step is optional.

Example 50: Preparation of 9-(2-(R)-benzyloxymethyl-1,3-dioxolan-4-yl)-6-chloro-2-amino purine (Compound 50a) and 9-(2-(R)-benzyloxymethyl-1,3-dioxolan-4-yl)-6-iodo-2-amino purine (Compound 50b).



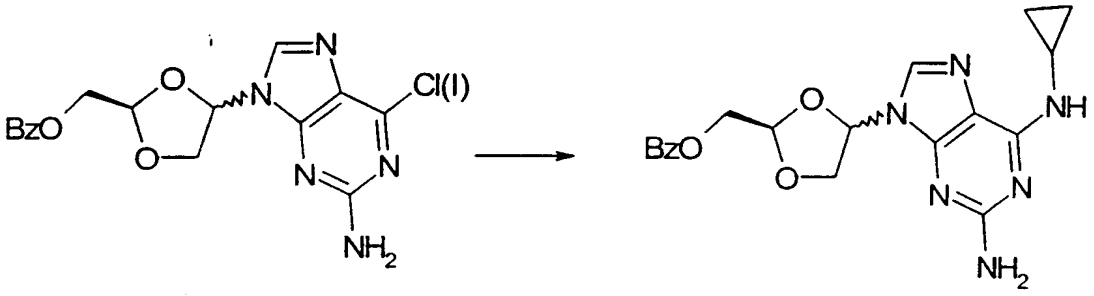
Compound 50a(b)

TMSI (28.2 mL; 198.12 mol eq) was added dropwise to a dichloromethane (750 mL) solution of the sugar (2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane) (52.75 g; 198.12 mmol; 1 eq) at -15°C. After 2.5 hr at -15°C, silylated 2-amino-6-chloropurine (62 g; 198 mmol; 1 eq) was added to the reaction mixture as a solid. The stirring was continued at the same temperature for another 2.5 hr. The reaction mixture was allowed to warm up slowly to room temperature followed by continued stirring for 40 hr at room temperature. Then, the mixture was poured onto aq $NaHCO_3$ solution (1 L). It was stirred for 20 min with $Na_2S_2O_3$ and filtered through a small pad celite. Then, the organic phase was separated and the aqueous phase was extracted with dichloromethane (1 X 200 mL). The combined organic phases were concentrated to get 87 g of the crude. Column purification of the crude on silica gel (450 g), eluting with ethylacetate/hexane (6:4) yielded 67.7 g (81%; 1:1 chloro/iodo mixture) of the coupled product with β/α ratio 2.3:1.

Alternatively, if the desired final product is the same compound but with opposite stereochemistry (i.e. a 2:1 mixture of β:α stereoisomers in the L-configuration).

The procedure discussed above is followed. However, the sugar 2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane is replaced with 2-(S)-benzyloxymethyl-4-carboxyl-1,3-dioxolane.

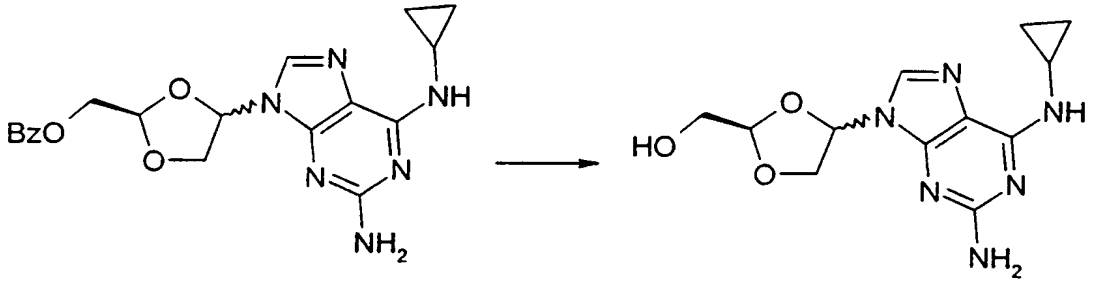
Example 51: Preparation of 9-(2-(R)-benzyloxymethyl-1,3-dioxolan-4-yl)-6-(N-cyclopropyl)amino-2-amino purine Compound 51).



Compound 51

A solution of the starting material (6.3 g; 14.95 mmol; 1 eq; average f.w.=421.52; Cl:I/1:1) in ethanol (100 mL) was refluxed at 75-80°C with cyclopropylamine (3.1 mL; 44.84 mmol; 3 eq) for 20 hrs and cooled to room temperature. The reaction mixture was concentrated, dissolved in dichloromethane (25 mL) and poured onto saturated aqueous sodium bicarbonate solution. After 10 min. of stirring, the organic phase was separated, and the aqueous phase was extracted with dichloromethane (2X15 mL). Then, the combined organic phase was concentrated to get a quantitative yield of the crude, which was then purified by column chromatography (silica gel, ethylacetate:MeOH 98.5:2.5 and 95:5) to yield 5.3 g (89%) of the product as a β/α mixture.

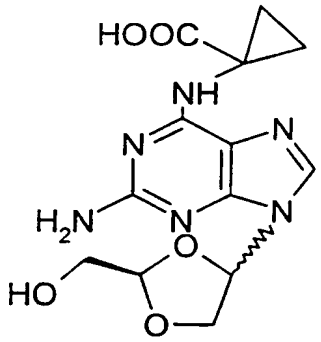
Example 52: Preparation of 9-(2-(R)-hydroxymethyl-1,3-dioxolan-4-yl)-6-(N-cyclopropyl)amino-2-amino purine (Compound 52).



Compound 52

The starting material (3.3 g) was stirred with ammonia in MeOH (80 mL; 2M) for 20 hrs. Nitrogen was bubbled through the reaction mixture to remove the excess ammonia. Then, the solution was concentrated to yield the crude as a β/α mixture (β/α = 2.3:1). The β/α isomers were separated by chromatography on silica gel using DCM/MeOH as eluent to yield 1.18 g (70% β isomer).

Example 53: Preparation of 9-(2-(R)-hydroxymethyl-1,3-dioxolan-4-yl-6-(N-2-cyclopropyl-2-aminomethoxyl)-2-amino purine (Compound 53).

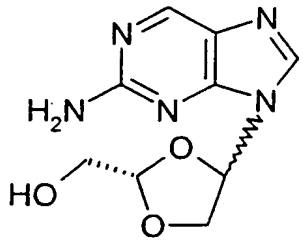


Compound 53

A solution of (2R)-2-benzoyloxymethyl-4-(2'-amino-6'-cyclopropylamino-purine-9'-yl)-1,3-dioxolane (480 mg) in 30 ml of saturated methanolic ammonia was stirred at room temperature for 18 h. The mixture was evaporated to dryness in vacuo. The residue was dissolved in 20 ml of water, washed twice with 10 ml of methylene chloride and lyophilized to give 283 mg of white solid in 80% yield. The resulting product had a mixture of β:α anomers having a ratio of about 2:1.

Alternatively, if the desired final product is the same compound but with opposite stereochemistry (i.e. a 2:1 mixture of β:α stereoisomers in the L-configuration). The procedure discussed above is followed. However, when following the steps of Example 50, the sugar 2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane is replaced with 2-(S)-benzyloxymethyl-4-carboxyl-1,3-dioxolane.

Example 54: Preparation of 9-(2-(S)-hydroxymethyl-1,3-dioxolan-4-yl)-2-amino purine (Compound 54).

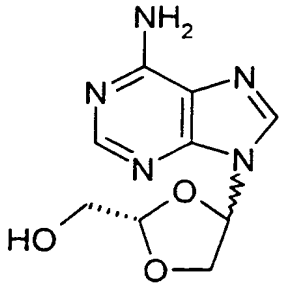


Compound 54

The procedure of Example 50 was performed. Thereafter 6.3 g of Compound 50 was subject to hydrogenation conditions under 50 psi of hydrogen over 10% Pd/c in 300 mL of ethanol containing 100 mL of triethylamine. After 3 hours of shaking, the catalyst was removed by filtration. Then the sovent was evaporated to yield a solid which was recrystallised to from ethanol-ether to give about 4 g of Compound 54 having about a 2:1 mixture of β:α stereoisomers in the L-configuration.

Alternatively, if the desired final product is the same compound but with opposite stereochemistry (i.e. about a 2:1 mixture of β:α stereoisomers in the D-configuration). The procedure discussed above is followed. However, when following the steps of Example 50, the sugar 2-(S)-benzyloxymethyl-4-carboxyl-1,3-dioxolane is replaced with 2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane.

Example 55: Preparation of 9-(2-(S) hydroxymethyl-1,3-dioxolan-4-yl)-6-amino purine (Compound 55).

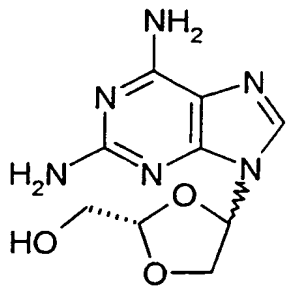


Compound 55

The procedures set forth in Examples 50 and 51 were performed. However, when following the steps of Example 50, the 1 equivalent of the silated 2-amino-6-chloropurine is replaced with 1 equivalent of silated 6-aminopurine. The result is a yield of 9-(2-(S)-hydroxymethyl-1,3-dioxolan-4-yl)-6-amino purine having a β:α ratio of about 2:1.

Alternatively, if the desired final product is the same compound but with opposite stereochemistry (i.e. a 2:1 mixture of β:α stereoisomers in the D-configuration). The procedure discussed above is followed. However, when following the steps of Example 50, the sugar 2-(S)-benzyloxymethyl-4-carboxyl-1,3-dioxolane is replaced with 2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane.

Example 56: Preparation of 9-(2-(S) hydroxymethyl-1,3-dioxolan-4-yl)-6,2-diamino purine (Compound 56).

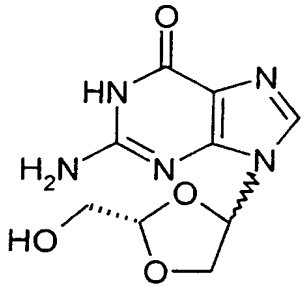


Compound 56

The procedure of Example 50 was performed. Thereafter, 6 g of Compound 50 was dissolved in 0.9 L of methanol saturated at 0°C with dry ammonia and the solution is heated in a steel bomb to 105°C to 110°C for 16 hours. The solution was evaporated to dryness and the residue purified by chromatography on silica gel using chloroform-methanol (4:1) as the eluent to give about 3g of crude Compound 56. The product can be recrystallised from methanol-ether to yield purified Compound 56 having a β:α ratio of about 2:1.

Alternatively, if the desired final product is the same compound but with opposite stereochemistry (i.e. a 2:1 mixture of β:α stereoisomers in the D-configuration). The procedure discussed above is followed. However, when following the steps of Example 50, the sugar 2-(S)-benzyloxymethyl-4-carboxyl-1,3-dioxolane is replaced with 2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane.

Example 57: Preparation of 9-(2-(S) hydroxymethyl-1,3-dioxolan-4-yl)-6-oxo-2-amino purine (Compound 57).

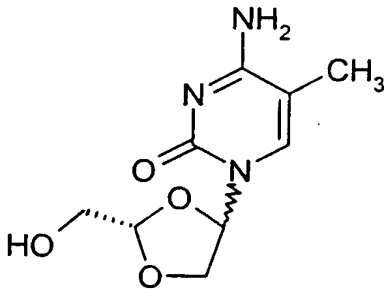


Compound 57

The procedure of Example 50 was performed. Thereafter, about 6 g of Compound 50 was dissolved in a mixture of 200 mL of methanol, 50 mL of water and 10 g of NaoH. The solution was heated under reflux for 5 hours after which time it was diluted with 300 mL of water and excess pyridinium sulfonate resin. The slurry was filtered, the resin washed with water and the combined aqueous filtrates were evaporated to dryness in vacuo to leave a residue which was taken up in 50% aqueous methanol. The solution was treated with activated charcoal, filtered and the filtrate evaporated to dryness in vacuo to give a solic residue that was recrystallized from ethanol water to yield pure compound 57 having a $\beta:\alpha$ ratio of about 2:1.

Alternatively, if the desired final product is the same compound but with opposite stereochemistry (i.e. a 2:1 mixture of $\beta:\alpha$ stereoisomers in the D-configuration). The procedure discussed above is followed. However, when following the steps of Example 50, the sugar 2-(S)-benzyloxymethyl-4-carboxyl-1,3-dioxolane is replaced with 2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane.

Example 58: Preparation of 9-(2-(S) hydroxymethyl-1,3-dioxolan-4-yl)-2-oxo-4-amino-5-methyl pyrimidine (Compound 58).

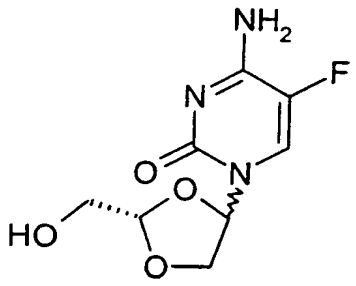


Compound 58

The procedure of Example 50 was performed followed by the procedure of Example 52. However, when following the steps of Example 50, the 1 equivalent of the silated 2-amino-6-chloropurine is replaced with 1 equivalent of silated 2-oxo-4-amino-5-methyl-pyrimidine. The result is a yield of 9-(2-(S)-hydroxymethyl-1,3-dioxolan-4-yl)-2-oxo-4-amino-5-methyl pyrimidine having a β:α ratio of about 2:1.

Alternatively, if the desired final product is the same compound but with opposite stereochemistry (i.e. a 2:1 mixture of β:α stereoisomers in the D-configuration). The procedure discussed above is followed. However, when following the steps of Example 50, the sugar 2-(S)-benzyloxymethyl-4-carboxyl-1,3-dioxolane is replaced with 2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane.

Example 59: Preparation of 9-(2-(S) hydroxymethyl-1,3-dioxolan-4-yl)-2-oxo-4-amino-5-fluoro pyrimidine (Compound 59).

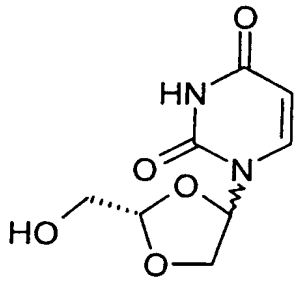


Compound 59

The procedure of Example 50 was performed followed by the procedure of Example 52. However, when following the steps of Example 50, the 1 equivalent of the silated 2-amino-6-chloropurine is replaced with 1 equivalent of silated 2-oxo-4-amino-5-fluoro-pyrimidine. The result is a yield of 9-(2-(S) hydroxymethyl-1,3-dioxolan-4-yl)-2-oxo-4-amino-5-fluoro pyrimidine having a β:α ratio of about 2:1.

Alternatively, if the desired final product is the same compound but with opposite stereochemistry (i.e. a 2:1 mixture of β:α stereoisomers in the D-configuration). The procedure discussed above is followed. However, when following the steps of Example 50, the sugar 2-(S)-benzyloxymethyl-4-carboxyl-1,3-dioxolane is replaced with 2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane.

Example 60: Preparation of 9-(2-(S) hydroxymethyl-1,3-dioxolan-4-yl)-2,4-dioxo pyrimidine (Compound 60).

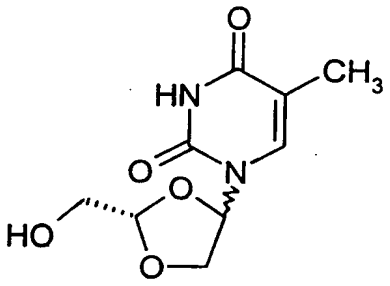


Compound 60

The procedure of Example 50 was performed followed by the procedure of Example 52. However, when following the steps of Example 50, the 1 equivalent of the silated 2-amino-6-chloropurine is replaced with 1 equivalent of silated 2,4-dioxo pyrimidine. The result is a yield of 9-(2-(S)-hydroxymethyl-1,3-dioxolan-4-yl)-2,4-dioxo pyrimidine having a β:α ratio of about 2:1.

Alternatively, if the desired final product is the same compound but with opposite stereochemistry (i.e. a 2:1 mixture of β:α stereoisomers in the D-configuration. The above formula is followed. However, when following the steps of Example 50, the sugar 2-(S)-benzyloxymethyl-4-carboxyl-1,3-dioxolane is replaced with 2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane.

Example 61: Preparation of 9-(2-(S) hydroxymethyl-1,3-dioxolan-4-yl)-2,4-dioxo-5-methyl pyrimidine (Compound 61).



Compound 61

The procedure of Example 50 was performed followed by the procedure of Example 52. However, when following the steps of Example 50, the 1 equivalent of the silated 2-amino-6-chloropurine is replaced with 1 equivalent of silated 2,4-dioxo-5-methyl pyrimidine. The result is a yield of 9-(2-(S) hydroxymethyl-1,3-dioxolan-4-yl)-2,4-dioxo-5-methyl pyrimidine having a β:α ratio of about 2:1.

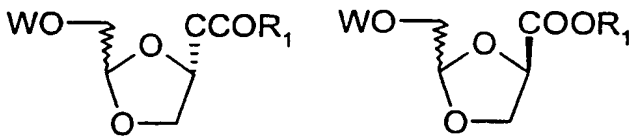
Alternatively, if the desired final product is the same compound but with opposite stereochemistry (i.e. a 2:1 mixture of β:α stereoisomers in the D-configuration). The procedure discussed above is followed. However, when following the steps of Example 50, the sugar 2-(S)-benzyloxymethyl-4-carboxyl-1,3-dioxolane is replaced with 2-(R)-benzyloxymethyl-4-carboxyl-1,3-dioxolane.

Some modifications and variations of the present invention including but not limited to selection of enzymes with high degree of sequence homology and optimization of reaction conditions will be obvious to a

person of ordinary skill in the art from the foregoing detailed description of the invention. Such modifications and variations are intended to fall within the scope of one or more embodiments of the present invention as defined by the following claims.

What is claimed is:

1. A process for stereoselectively producing a dioxolane nucleoside analogue from an anomeric mixture of β and α anomers represented by the following formula A or formula B:



(A) (B)

wherein W is benzyl or benzoyl and R_1 is selected from the group consisting of C_{1-6} alkyl and C_{6-15} aryl, the process comprising:

stereoselectively hydrolysing said mixture with an enzyme selected from the group consisting of cholesterol esterase, ESL-001-02, horse liver esterase, bovine pancreatic protease, α-chymotrypsin, protease from *Streptomyces caespitosis*, substilisin from *Bacillus licheniformis*, protease from *Aspergillus oryzae*, proteinase from *Bacillius licheniformis*, protease from *Streptomyces griseus*, acylase from *Aspergillus melleus*, proteinase from *Bacillus subtilis*, ESL-001-05, pronase protease from *Streptomyces griseus*, *lipase* from *Rhizopus arrhizus*, lipoprotein lipase from *Pseudomonas* species type B, lipase from *Pseudomonas cepacia* and bacterial proteinase to stereoselectively hydrolyse predominantly one anomer to form a product wherein R_1 is replaced with H;

separating the product from unhydrolysed starting material;

stereoselectively replacing the functional group at the C4 position ($COOR_1$) with a purinyl or pyrimidinyl or analogue or derivative thereof.

2. The process of claim 1, wherein the step of hydrolysing results in the starting material having an anomeric purity of at least 80%.

3. The process of claim 1, wherein the step of hydrolysing results in the starting material having an anomeric purity of at least 90%.

4. The process of claim 1, wherein the step of hydrolysing results in the starting material having an anomeric purity of at least 95%.

5. The process of claim 1, wherein the step of hydrolysing results in the starting material having an anomeric purity of at least 98%.

6. The process of claim 1, wherein the step of hydrolysing results in the product having an anomeric purity of at least 80%.

7. The process of claim 1, wherein the step of hydrolysing results in the product having an anomeric purity of at least 90%.

8. The process of claim 1, wherein the step of hydrolysing results in the product having an anomeric purity of at least 95%.

9. The process of claim 1, wherein the step of hydrolysing results in the product having an anomeric purity of at least 98%.

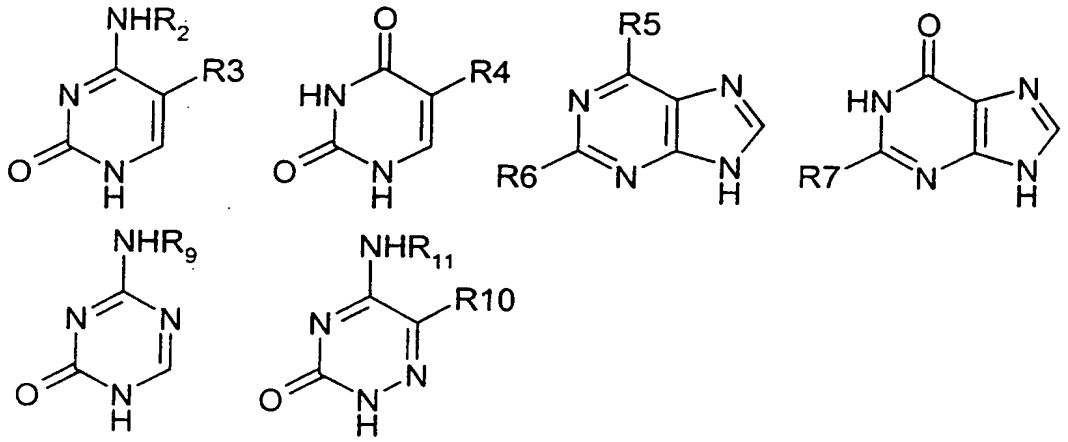
10. The process of claim 1, wherein W is benzyl and wherein the enzyme is selected from the group consisting of cholesterol esterase, ESL-001-02, horse liver esterase, bovine pancreatic protease, α-chymotrypsin, protease from *Streptomyces caespitosis*, substilisin from *Bacillus licheniformis*.

11. The process of claim 10, wherein the enzyme is α-chymotrypsin.

12. The process of claim 10, wherein the enzyme is bovine pancreatic protease.

13. The process of claim 1, wherein W is benzoyl and wherein the enzyme is selected from the group consisting of protease from *Aspergillus oryzae*, proteinase from *Bacillus licheniformis*, subtilisin from *Bacillus licheniformis*, protease from *Streptomyces griseus*, acylase from *Aspergillus melleus*, proteinase from *Bacillus subtilis*, ESL-001-05, pronase protease from *Streptomyces griseus*, lipase from *Rhizopus arrhizus*, lipoprotein lipase from *Pseudomonas* species type B, bacterial proteinase, lipase from *Pseudomonas cepacia*.

14. The process of claim 1, wherein the purinyl or pyrimidinyl or analogue or derivative thereof is selected from the group consisting of:



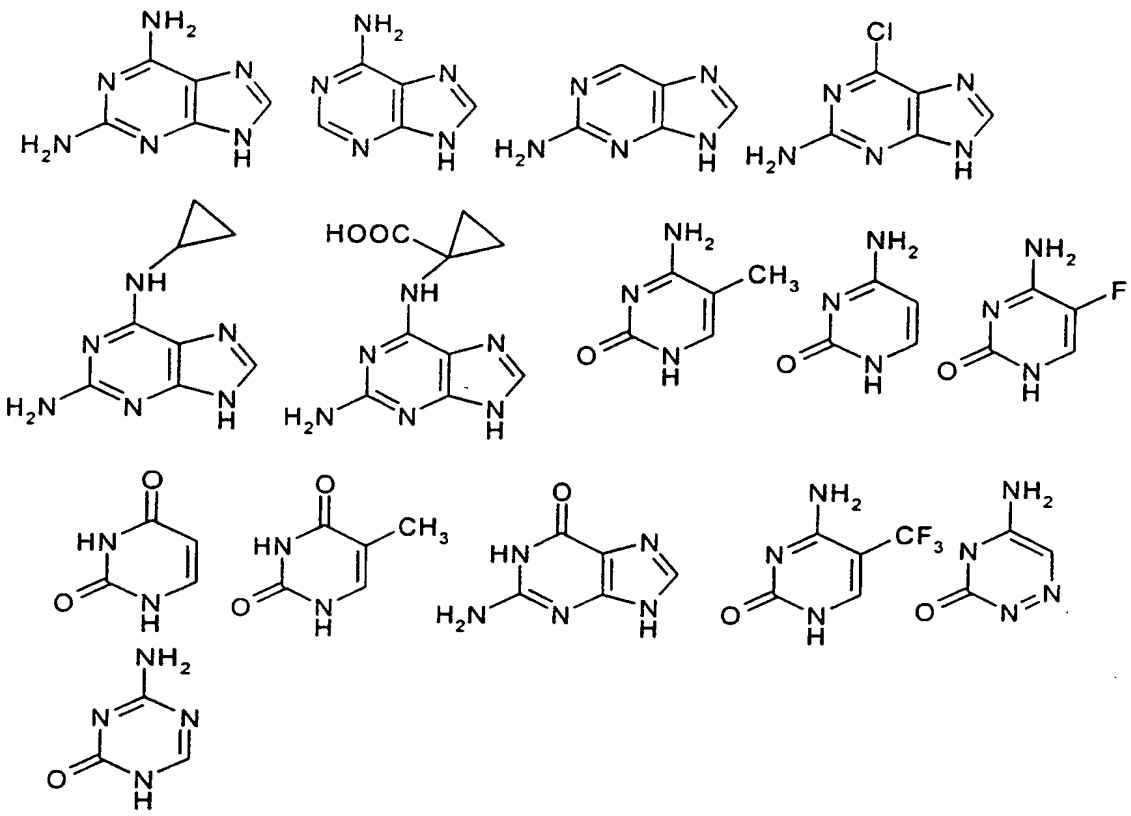
wherein

R_2, R_9 and R_{11} are selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6} acyl and $R_8C(O)$ wherein R_8 is hydrogen or C_{1-6} alkyl;

R_3, R_4 and R_{10} are each independently selected from the group consisting of hydrogen, C_{1-6} alkyl, bromine, chlorine, fluorine, iodine and CF_3; and

R_5, R_6 and R_7 are each independently selected from the group consisting of hydrogen, bromine, chlorine, fluorine, iodine, amino, hydroxyl and C_{3-6} cycloalkylamino.

15. The process of claim 1, wherein the purine or pyrimidine base or analogue or derivative thereof is selected from the group consisting of:



16. The process of claim 1, wherein the step of replacing further comprises:

acylating the second mixture to produce an acylated second mixture; and

glycosylating the acetylated second mixture with a purine or pyrimidine base or analogue or derivative thereof and a Lewis Acid to produce the dioxolane nucleoside analogue.

INTERNATIONAL SEARCH REPORT

Inte. onal Application No
PCT/CA 00/00144

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12P41/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 276 151 A (DENNIS C. LIOTTA) 4 January 1994 (1994-01-04) column 11, line 31 -column 13, line 46	1-16
A	WO 97 21706 A (BIOCHEM PHARMA INC.) 19 June 1997 (1997-06-19) cited in the application page 5, line 31 -page 6, line 19	1-16
A	DD 277 698 A (VE FORSCHUNGSZENTRUM BIOTECHNOLOGIE BERLIN) 11 April 1990 (1990-04-11) the whole document	1-13
A	US 5 190 867 A (MAURO A. BERTOLA ET AL.) 2 March 1993 (1993-03-02) column 1, line 48 -column 4, line 28	1-13

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
18 May 2000	13/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Montero Lopez, B

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